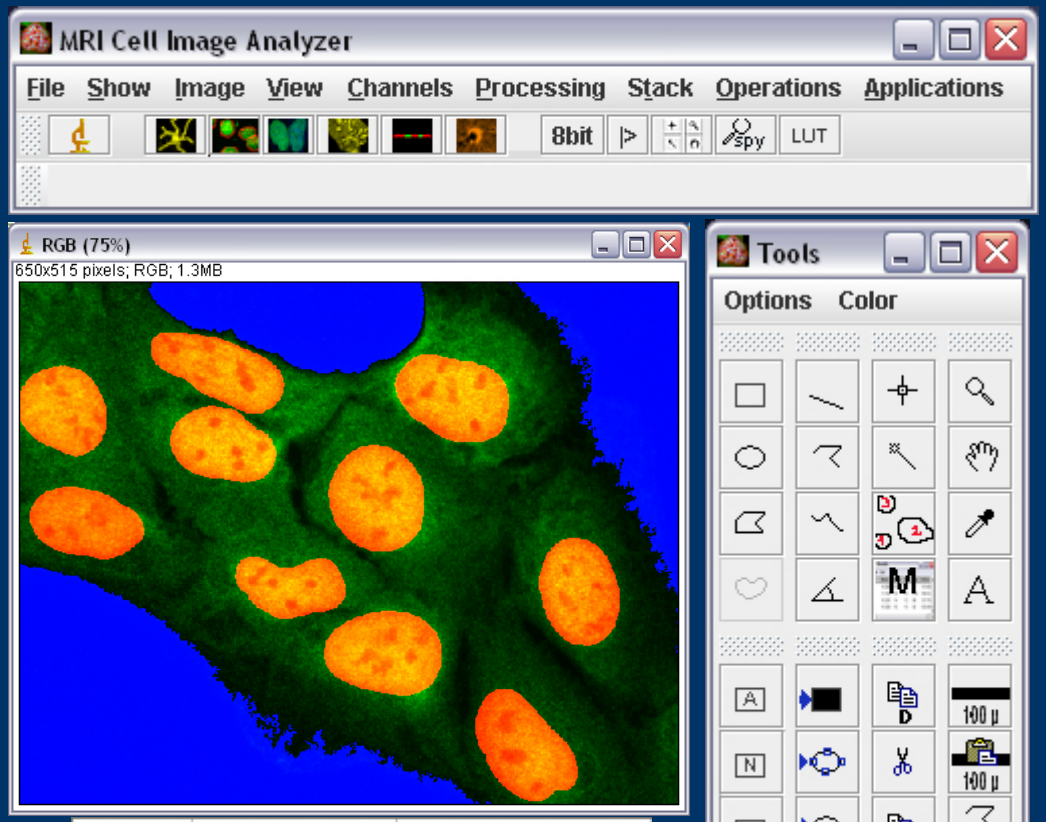
MRI Cell Image Analyzer - Automatic analysis of microscopy images



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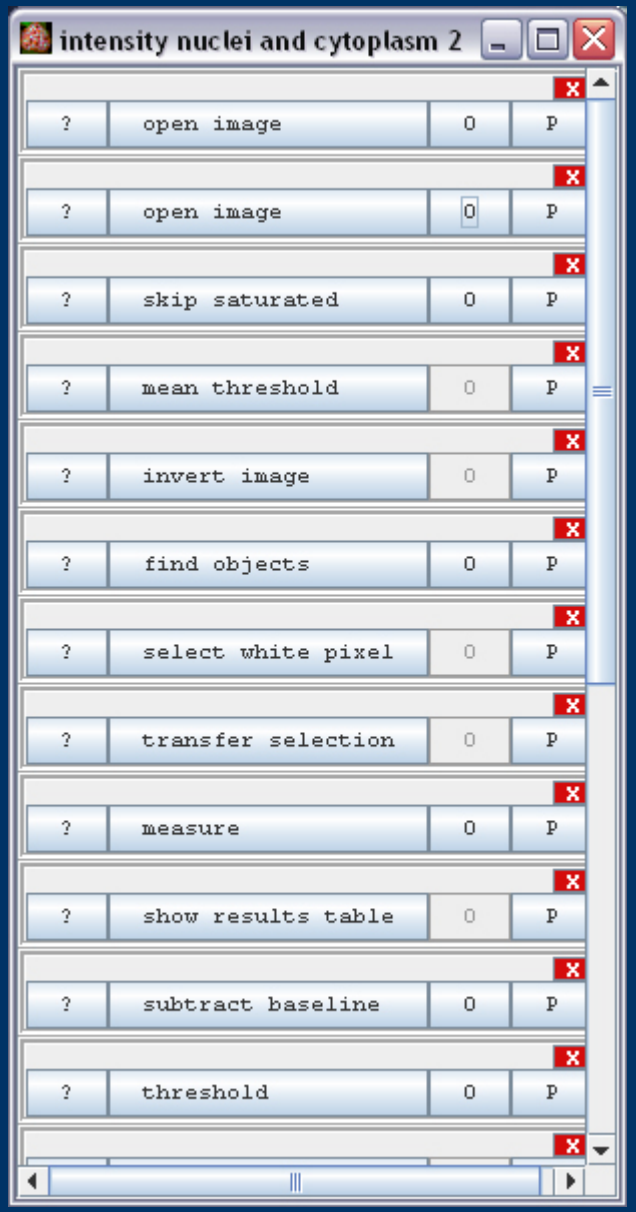

13.12.2005

Montpellier RIO Imaging
Volker Bäcker

volker.baecker@mri.cnrs.fr

MRI Cell Image Analyzer - overview

- project description
- application prototyping framework
- interactive tools
- basic image processing and analysis
- implemented projects (examples)
  - counting and measuring stained regions in cells
  - dna combing
  - neurite tracing and quantification (adaption of *NeuronJ*)
  - comparing intensities
  - counting cells
- summary and outlook

project description

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MRI Cell Image Analyzer - project description – the problem

- manual analysis of images
  - a time consuming task (think of robotized acquisition)
  - results may be involuntary biased and not reproducible
- general purpose tools
  - are often not apt for the automation of a specific task
    - no a priori knowledge about the contents of your images
  - they are not extendable
    - missing operations can only be added as a combination of existing operations

MRI Cell Image Analyzer - project description – the solution

1. a rapid prototyping framework for image analysis applications

- Requirements
  - allow interactive experimentation to find solutions
  - build applications from existing operations rapidly
  - add operations on the basic level when needed
  - applications must be usable by non computer specialist

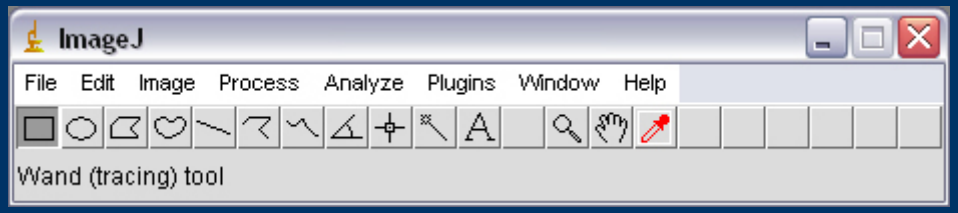
2. building applications on demand together with the scientist

3. expanding the framework as needed

MRI Cell Image Analyzer - project description – design decision

don't reinvent the wheel !

- base MRI-CIA on which image analysis library / kit ?
- ImageJ, because (Wayne Rasband, National Institute of Mental Health, Bethesada, Maryland, USA)
  - it has been created for the treatment of microscopy images
  - provides a solid image processing/analysis framework
  - an abundance of plugins for specific tasks available
  - a vivid user community
  - good documentation
  - it is public domain



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application prototyping framework

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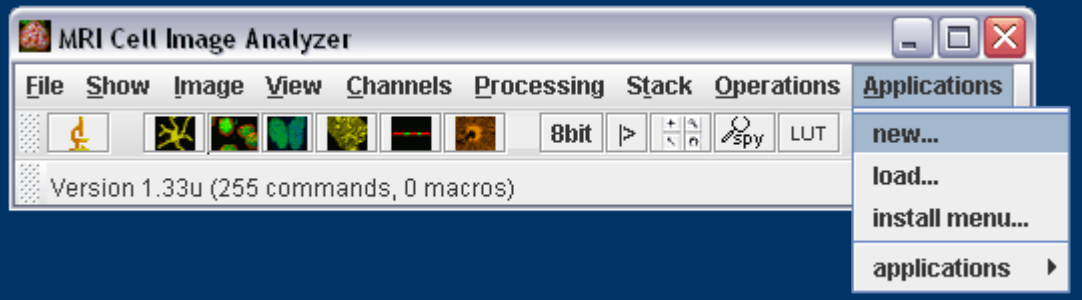
MRI Cell Image Analyzer - application prototyping framework

- build applications by connecting operations
- simple example



  - task:
    - convert images to 8 bit and enhance the contrast

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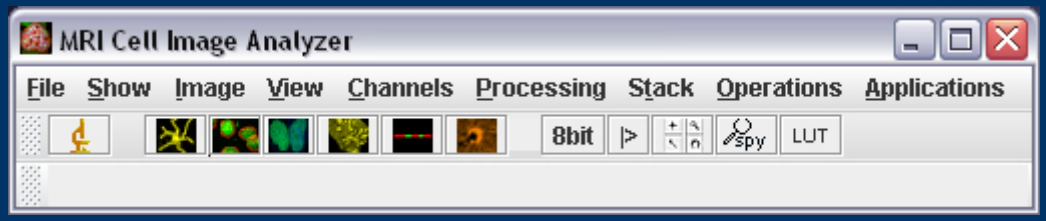

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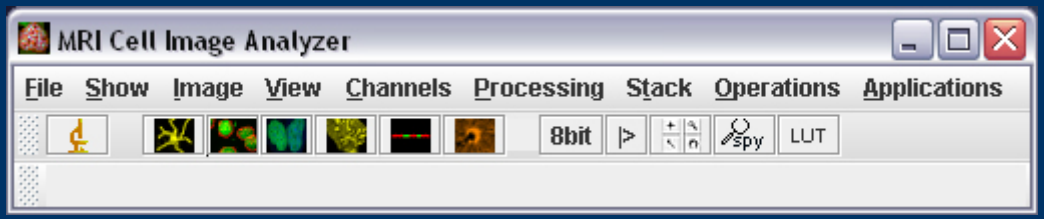

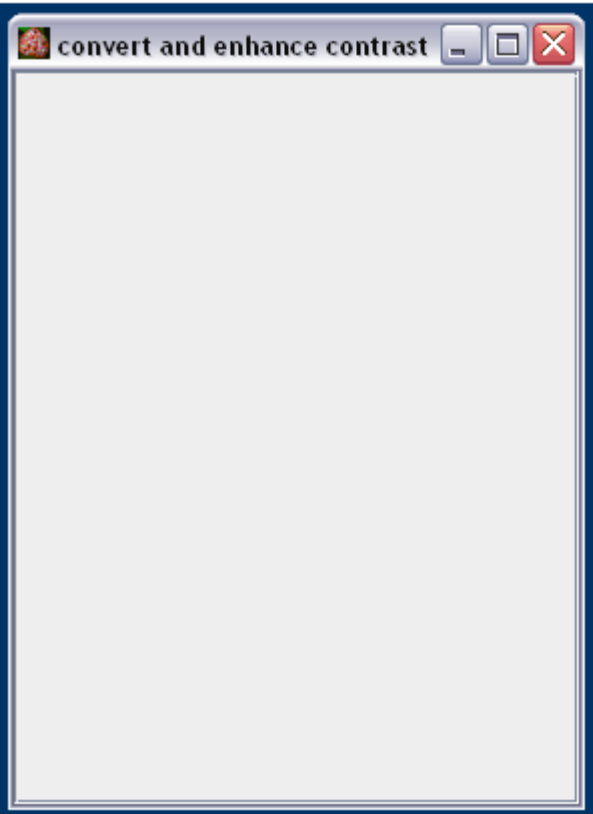

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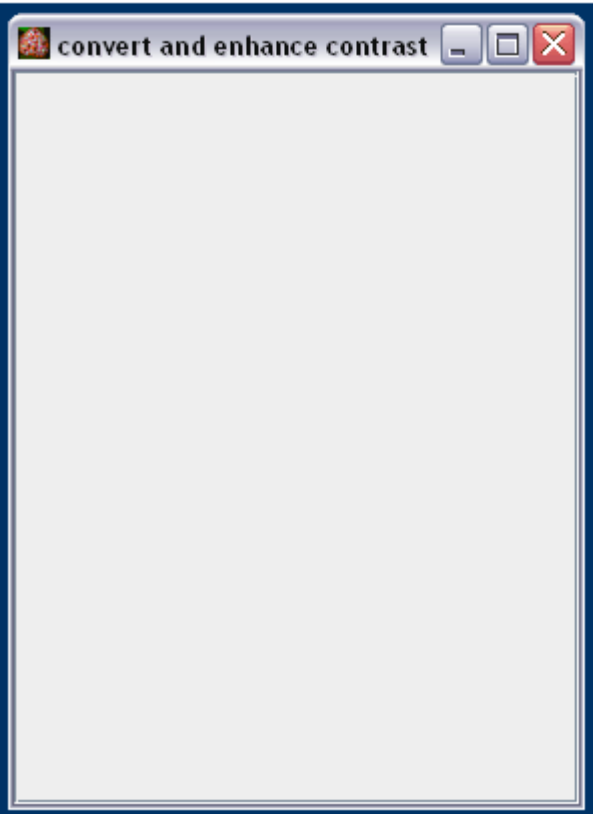

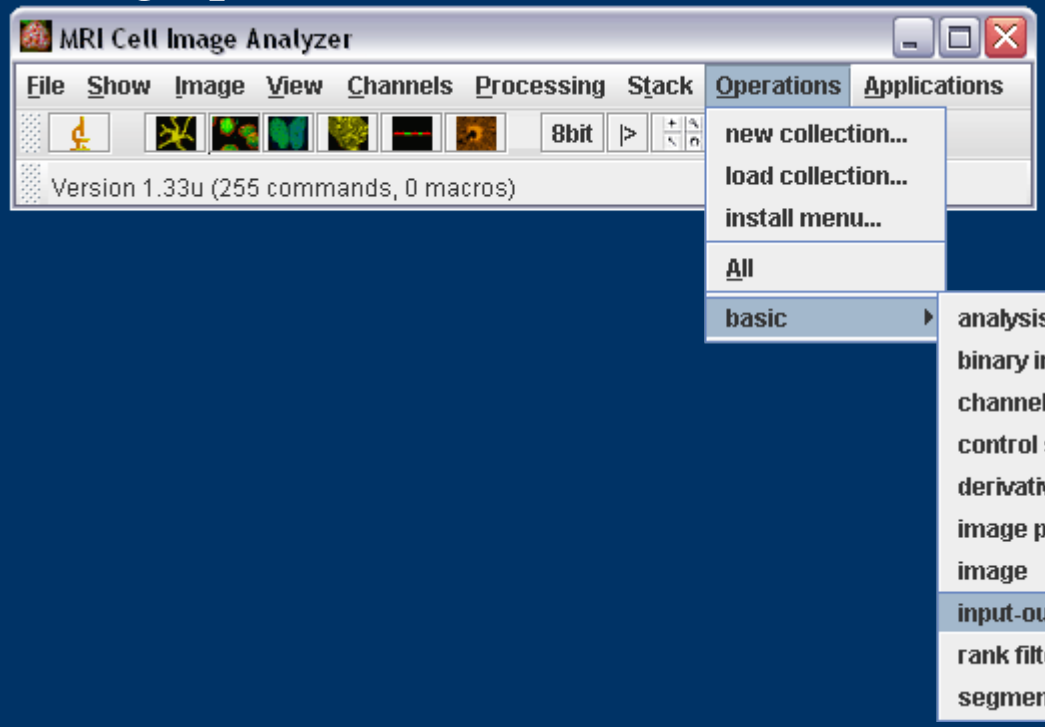

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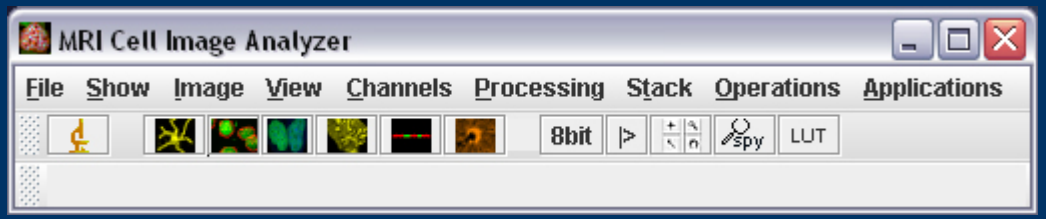

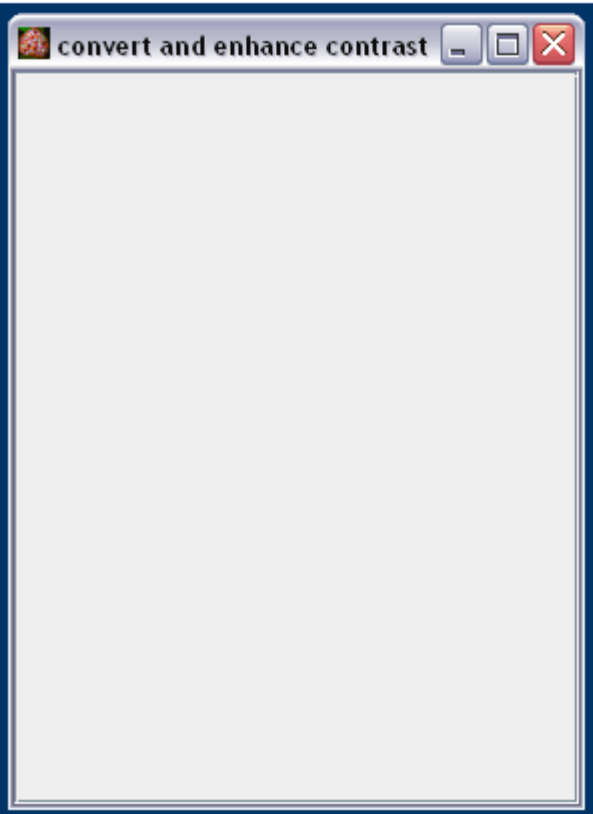

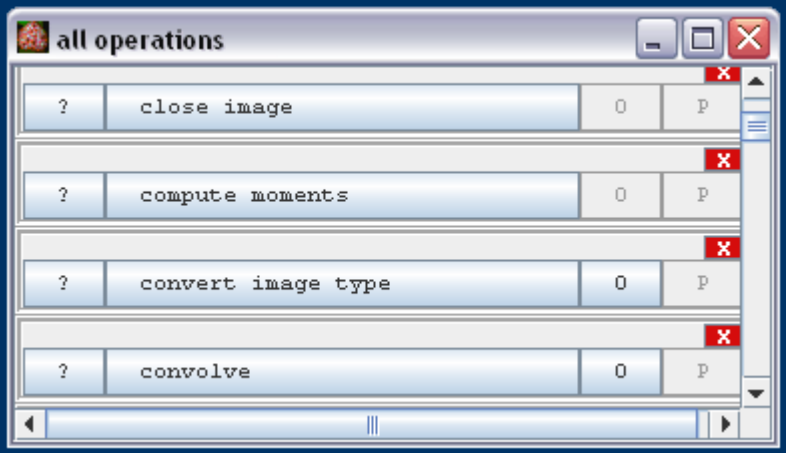

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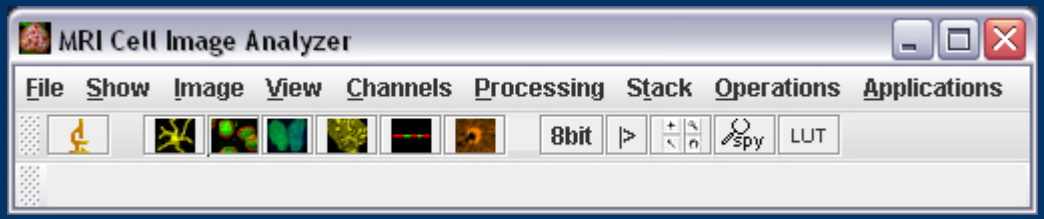

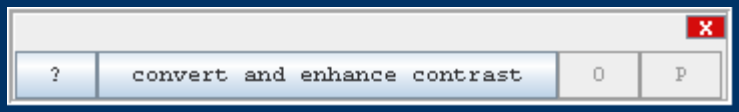

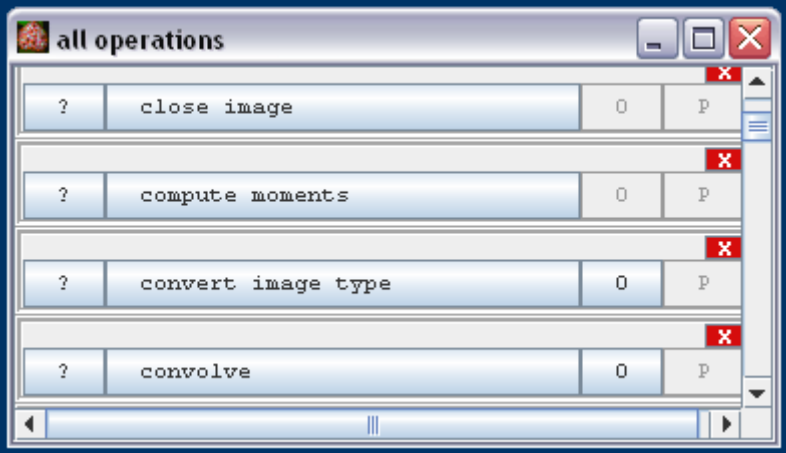

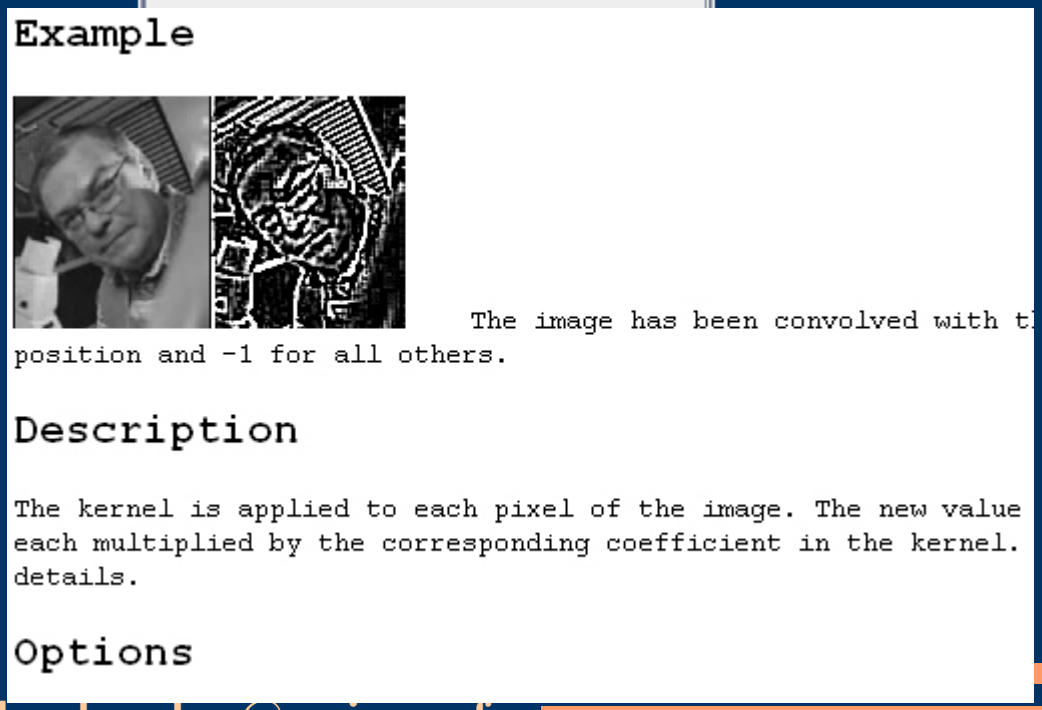
Example

The image has been convolved with t
position and -1 for all others.

Description

The kernel is applied to each pixel of the image. The new value
each multiplied by the corresponding coefficient in the kernel.
details.

Options

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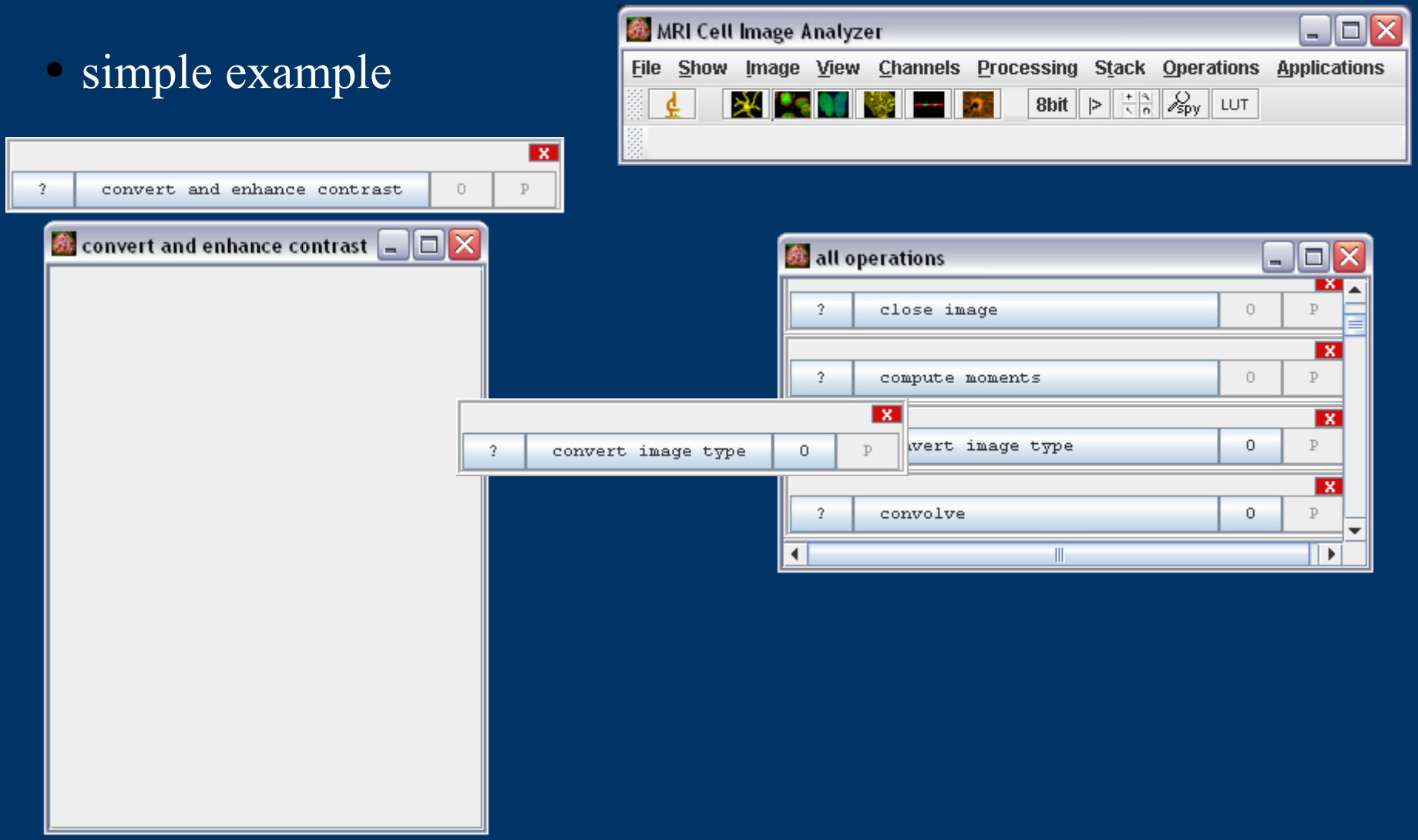

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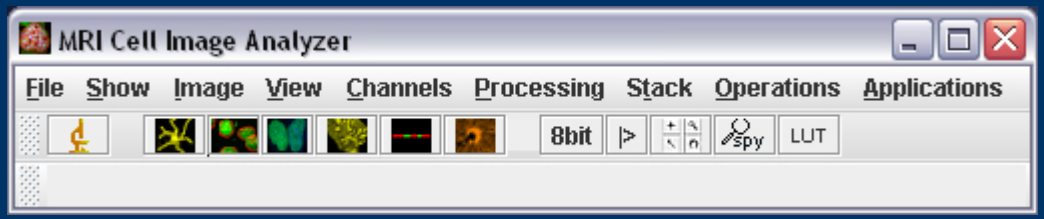

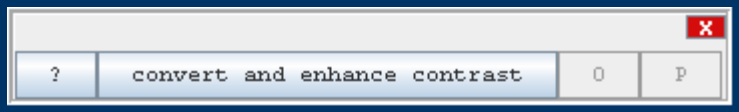

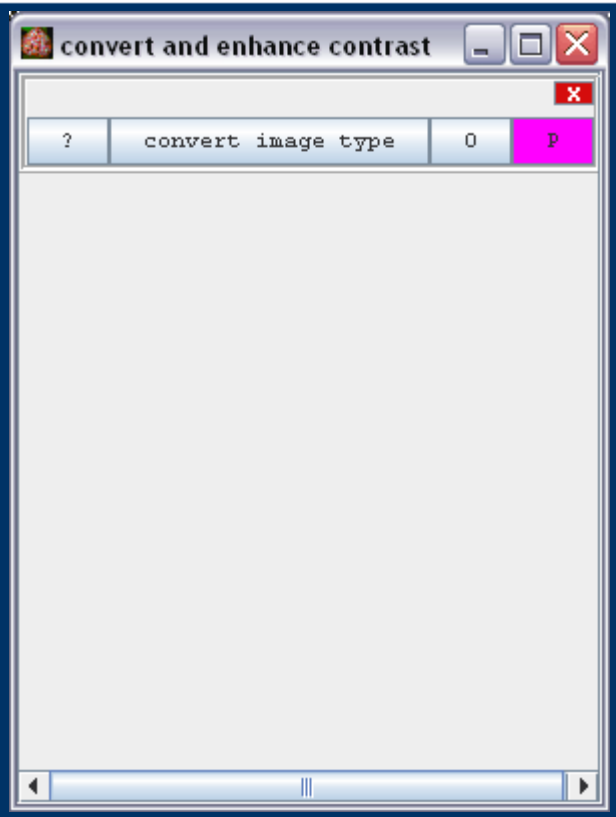

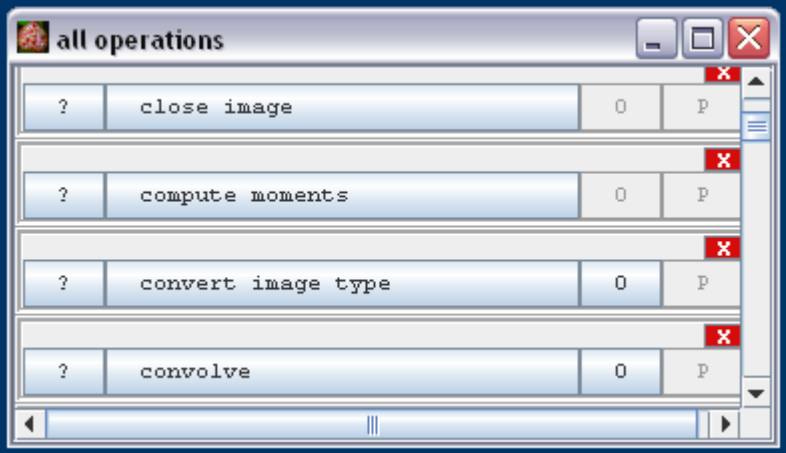

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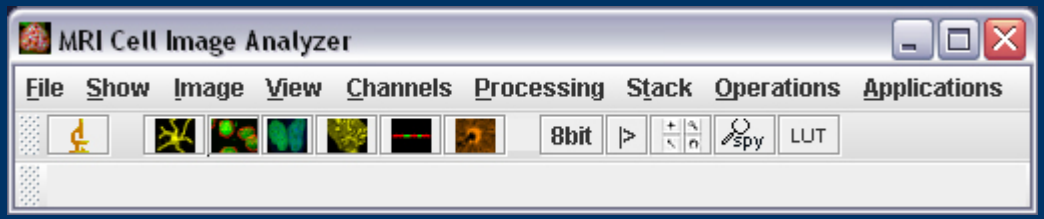

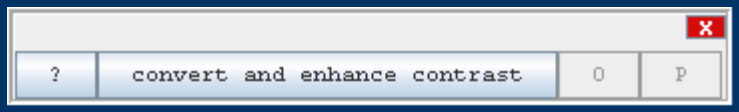

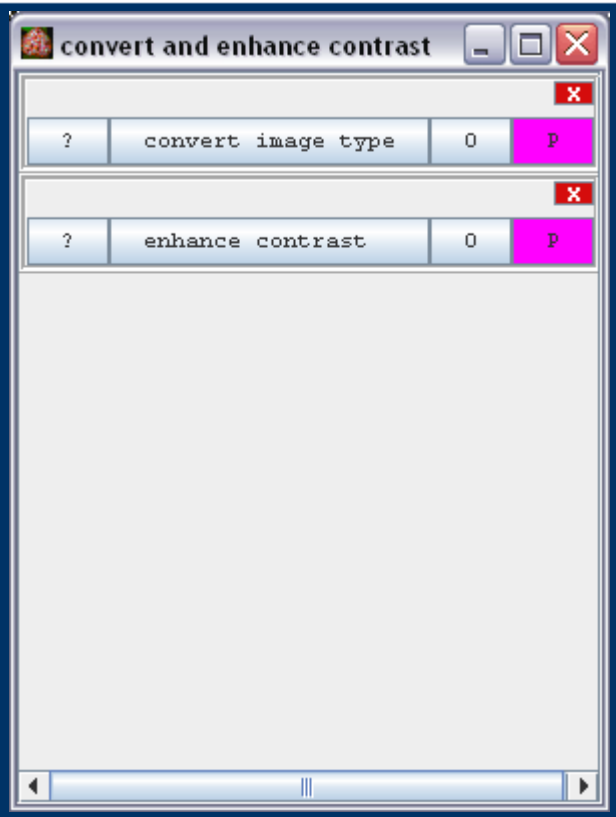

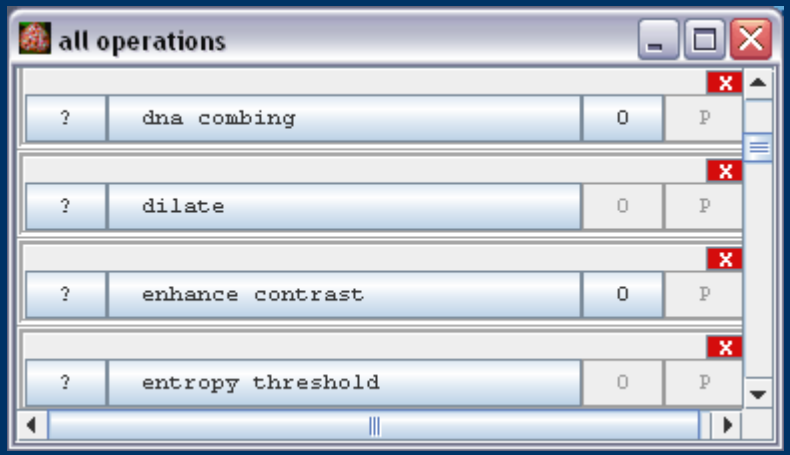

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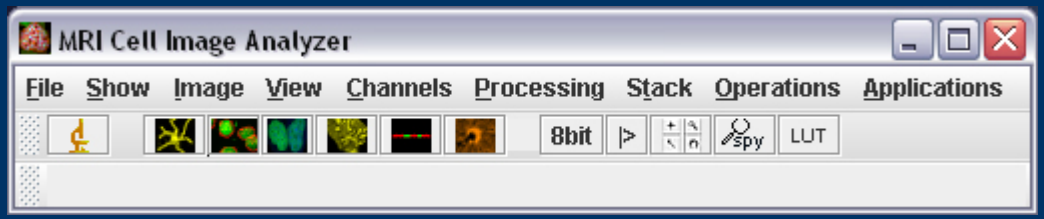

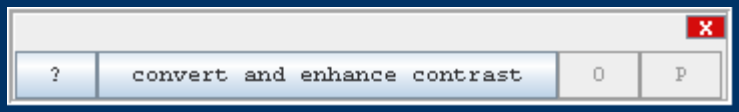

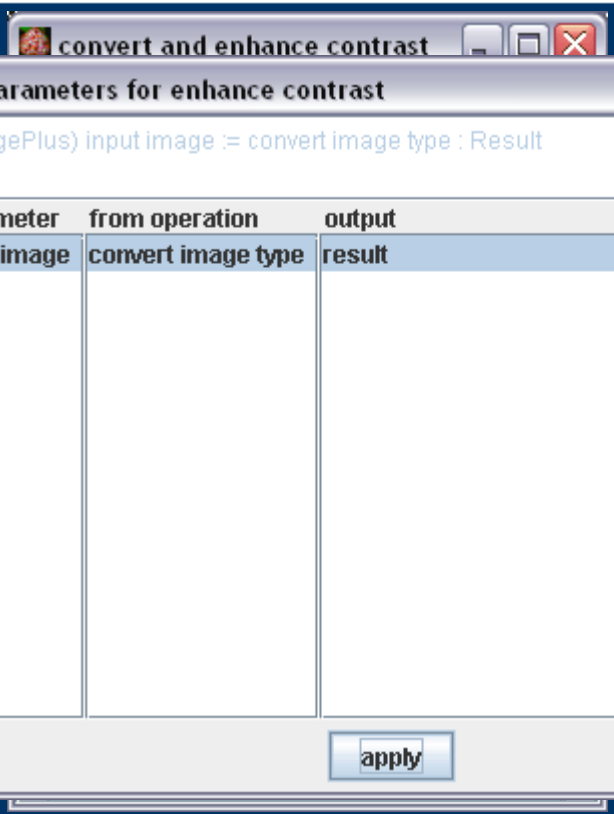

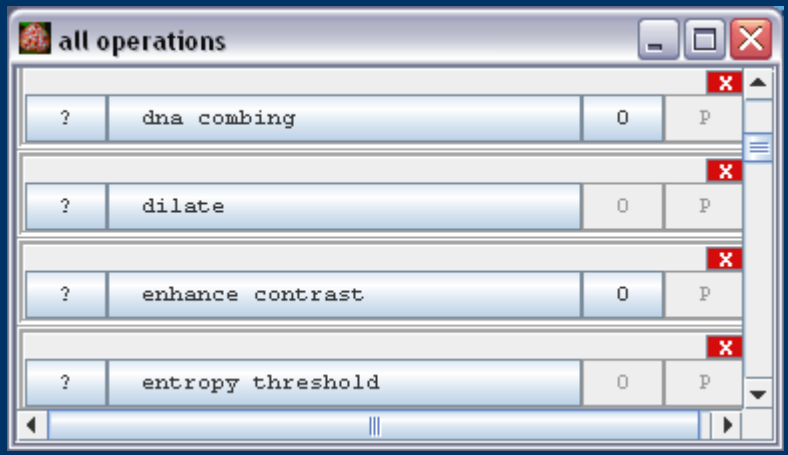

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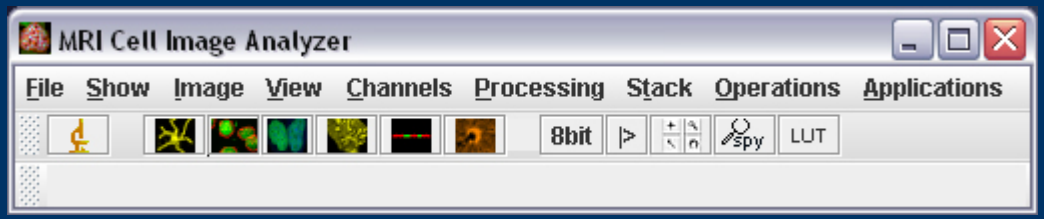

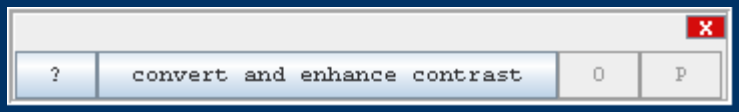

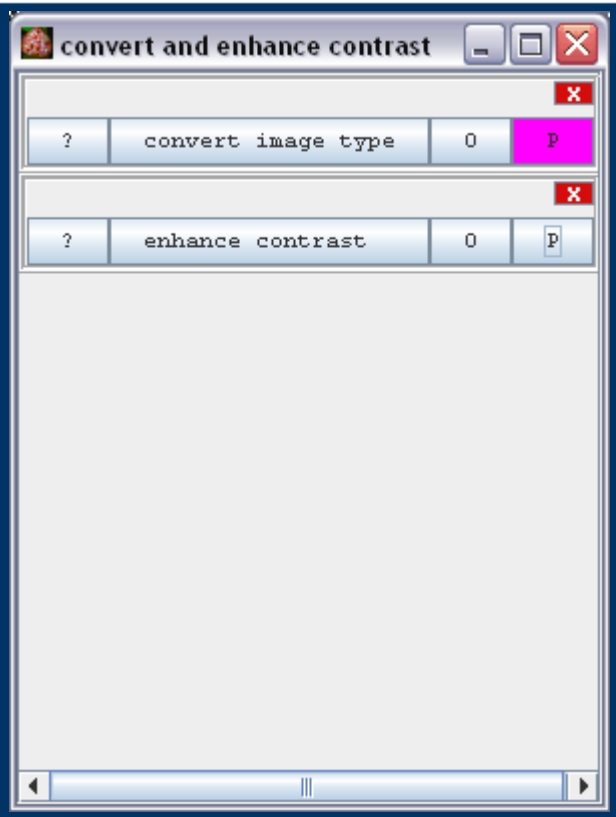

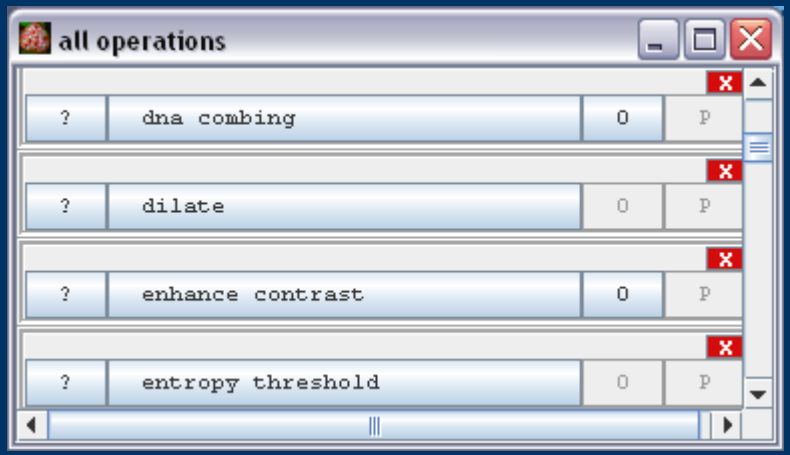

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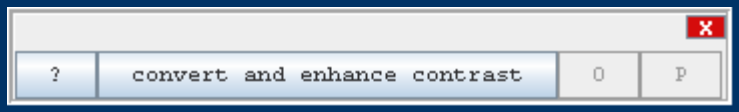

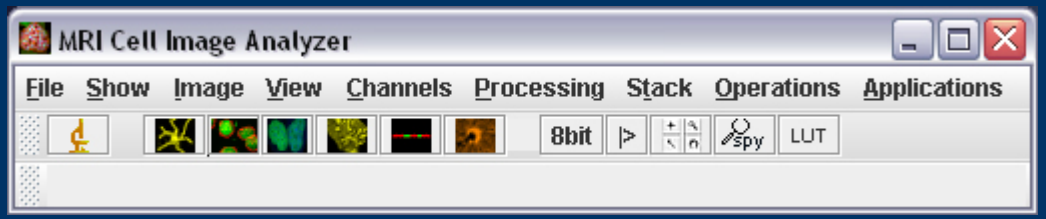

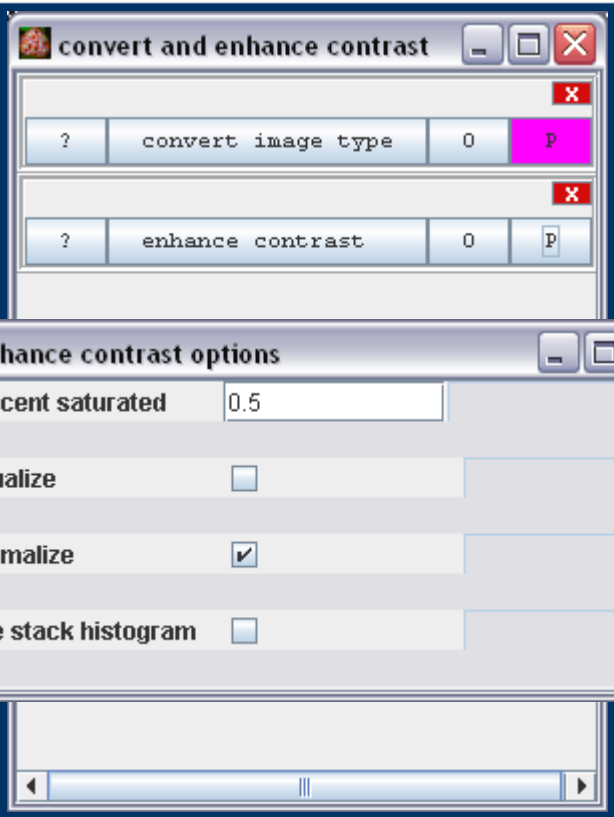

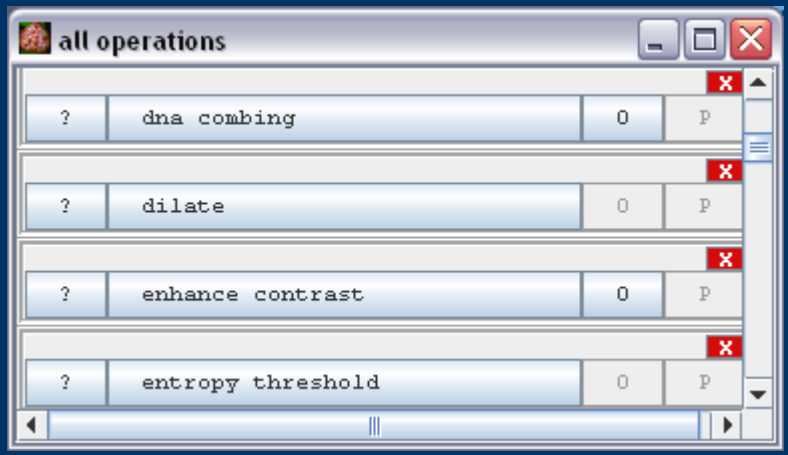

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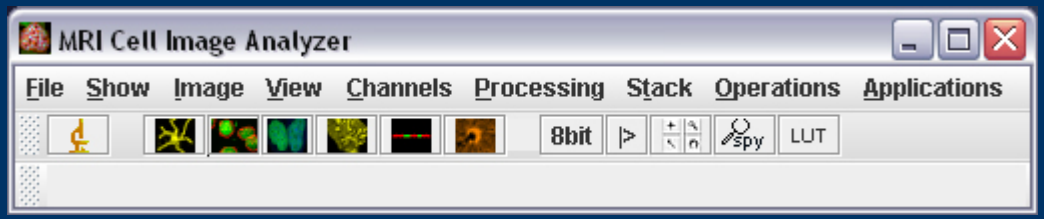

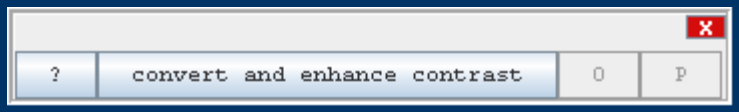

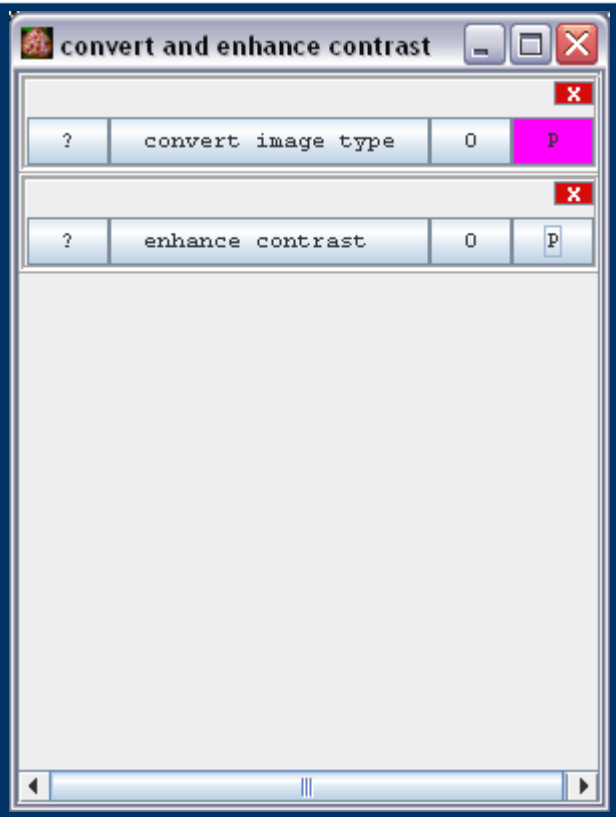

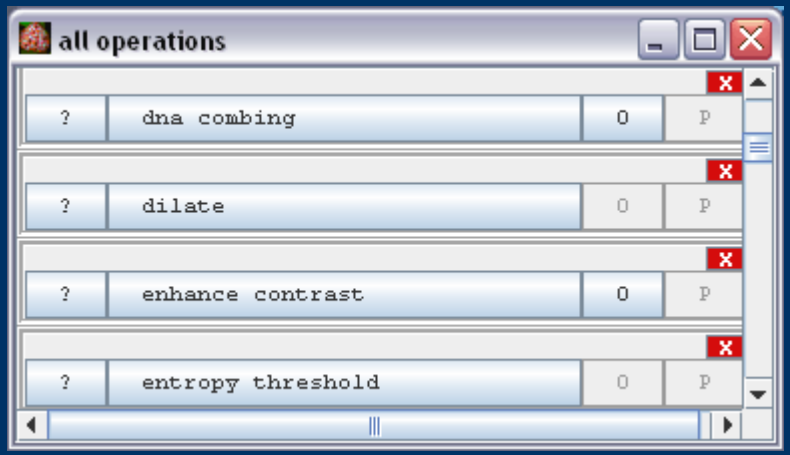

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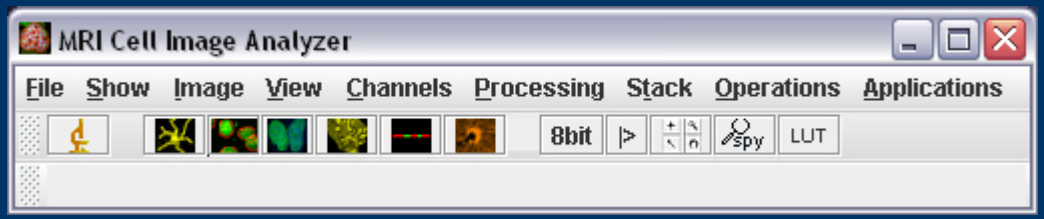

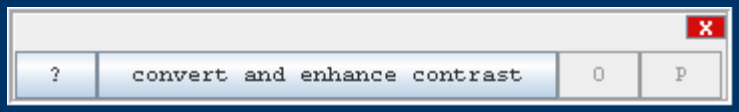

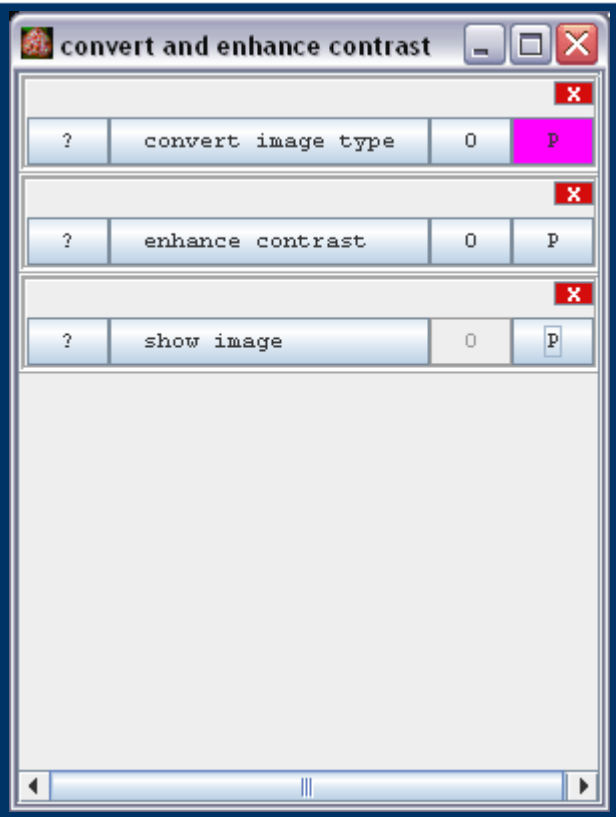

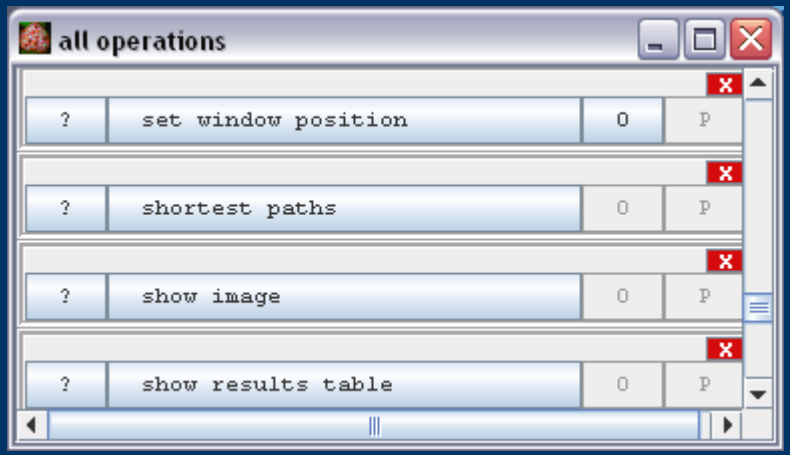

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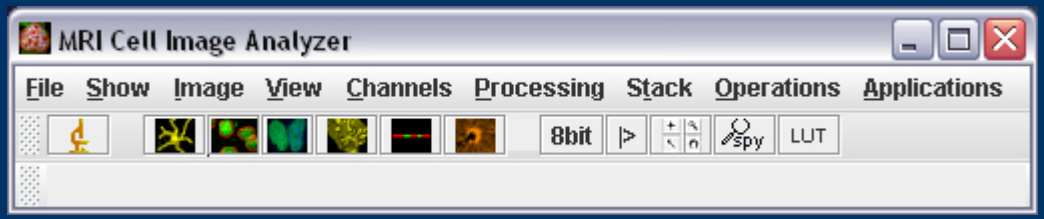

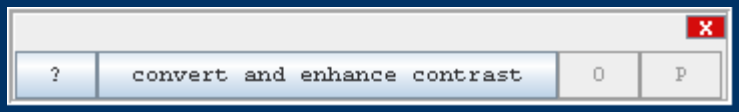

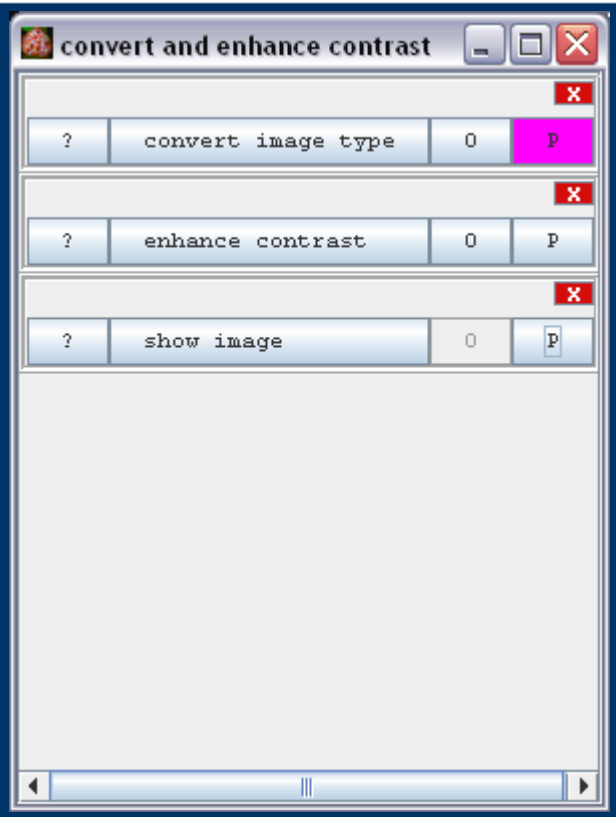

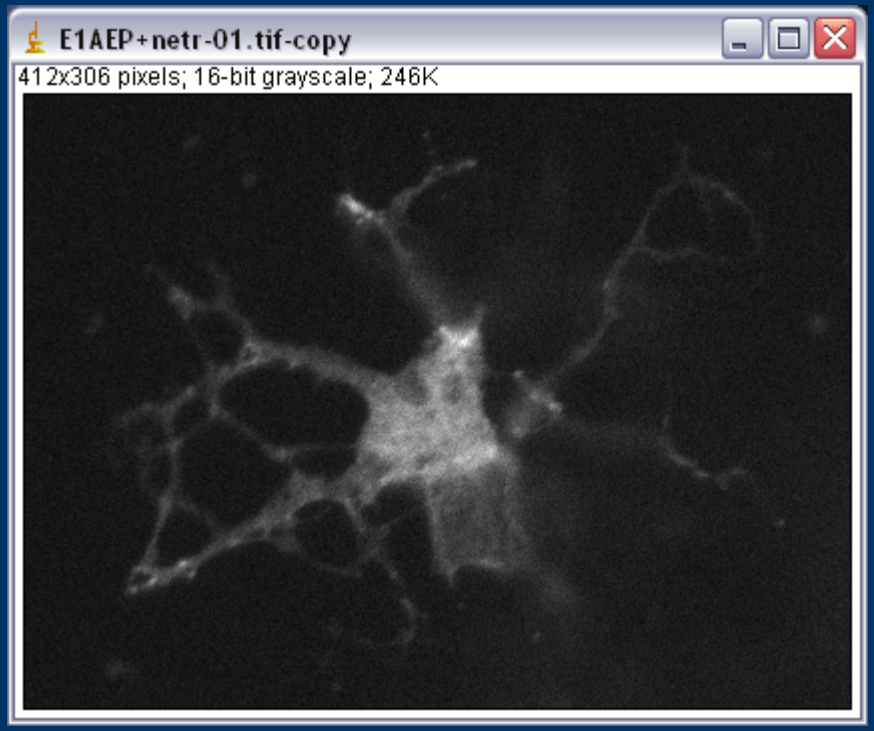

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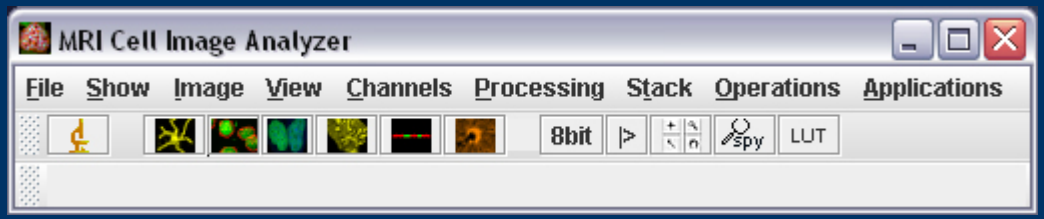
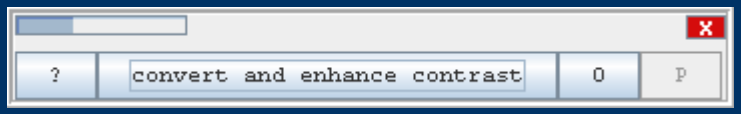
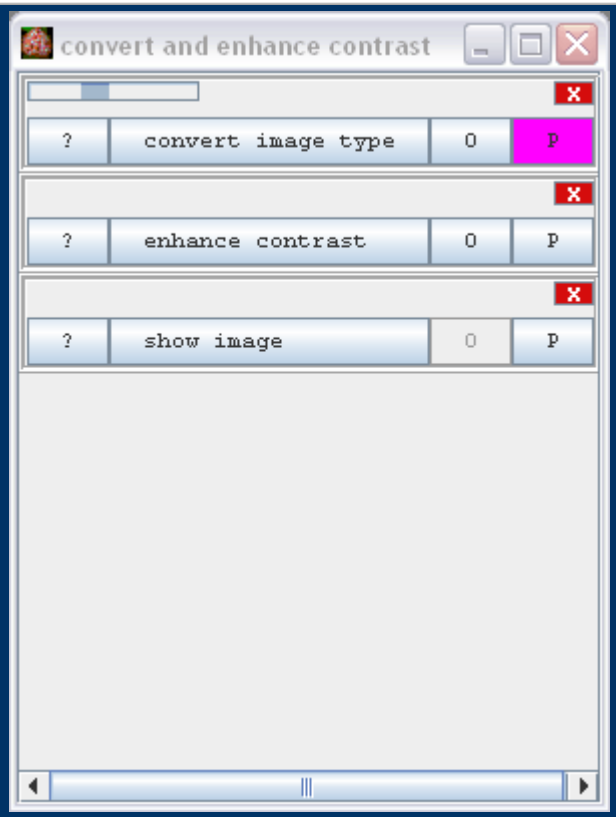
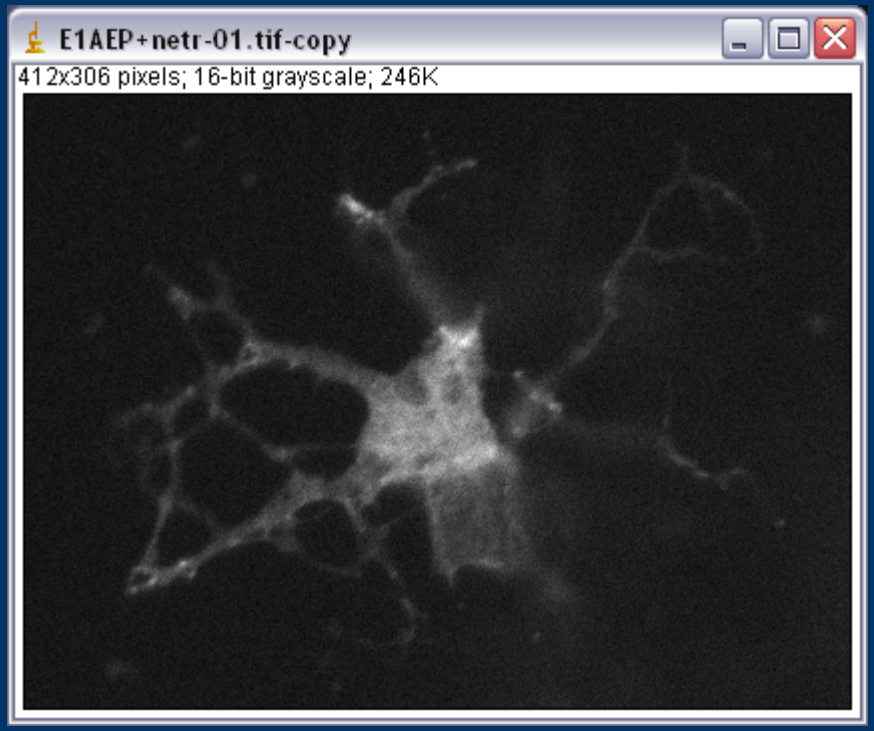
MRI Cell Image Analyzer - application prototyping framework

- build applications by connecting operations
- simple example









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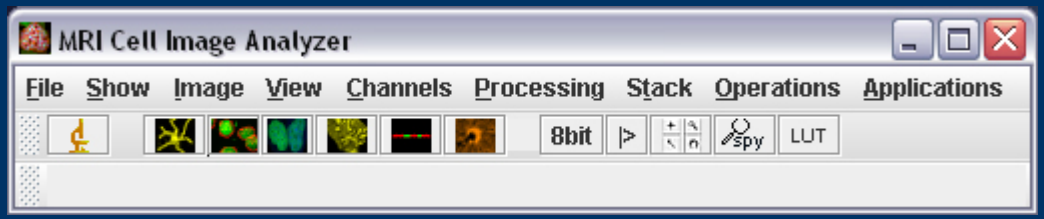
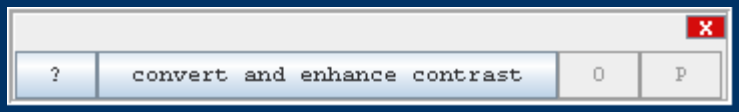
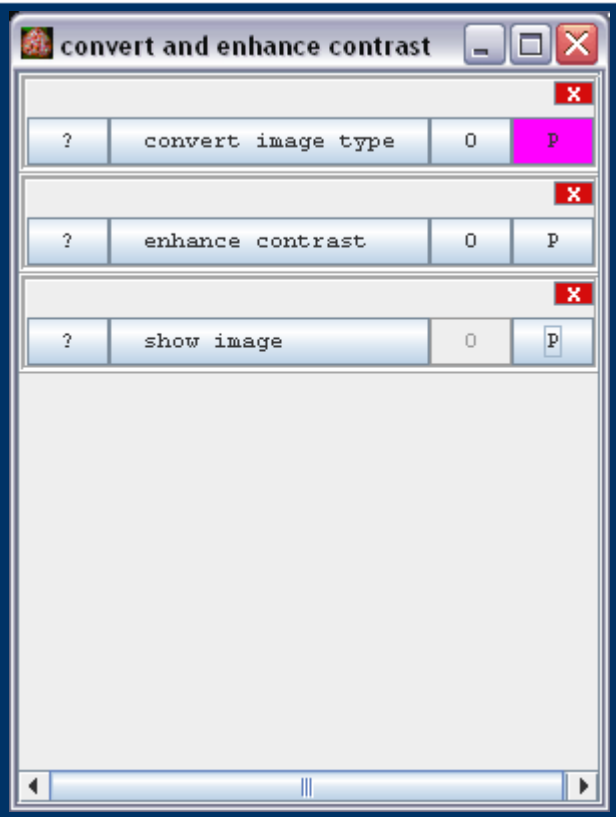
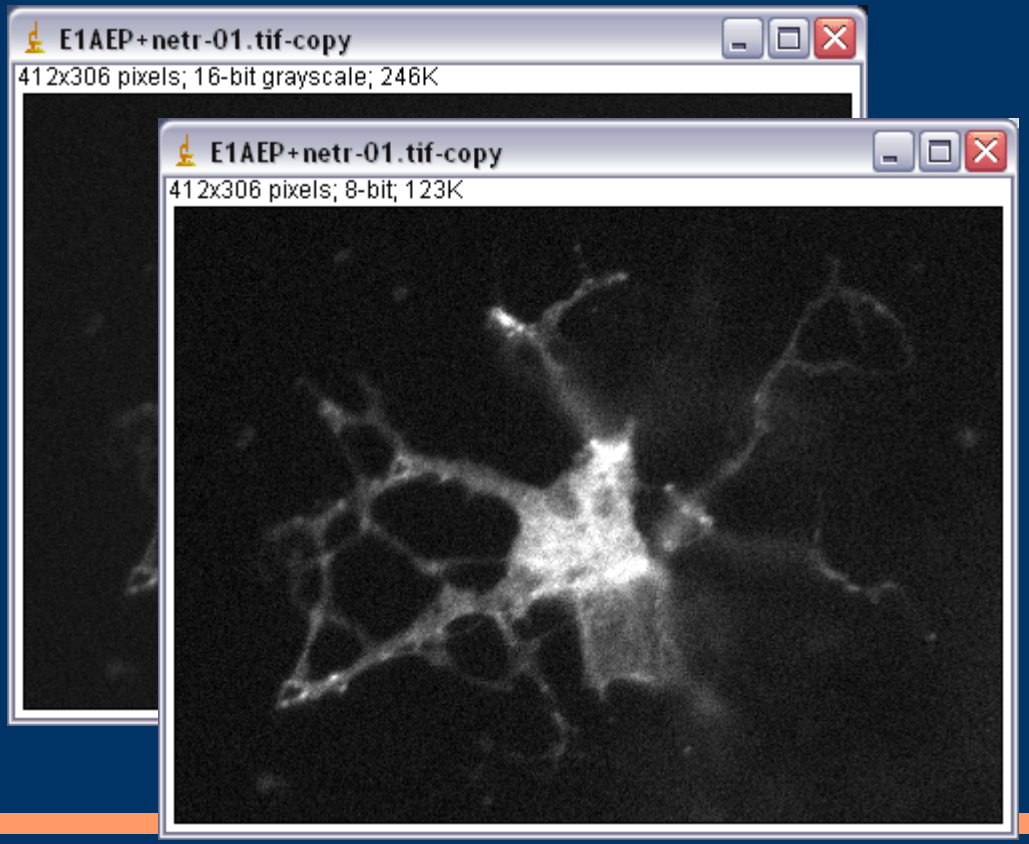
MRI Cell Image Analyzer - application prototyping framework

- build applications by connecting operations
- simple example









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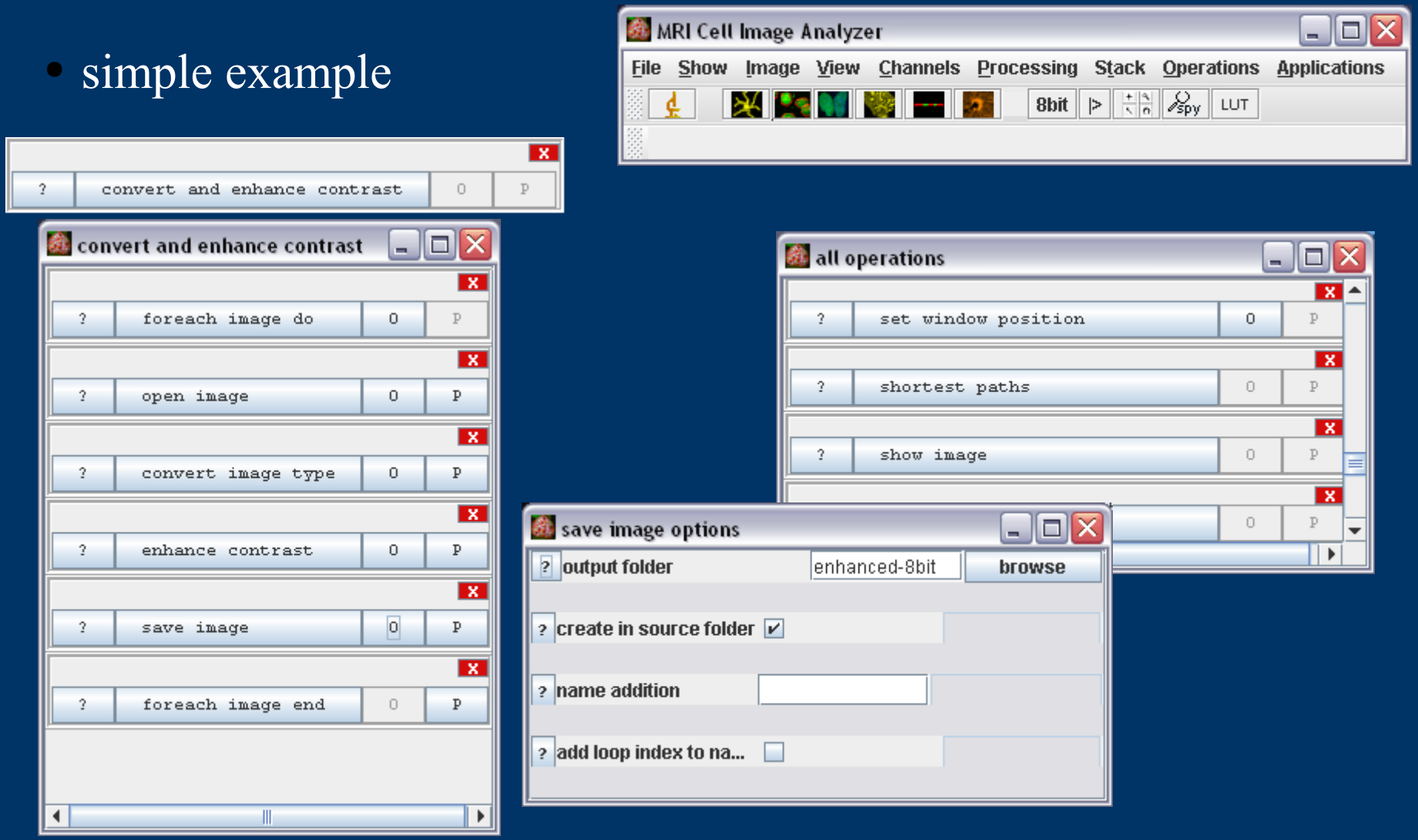
MRI Cell Image Analyzer - application prototyping framework

- build applications by connecting operations
- simple example



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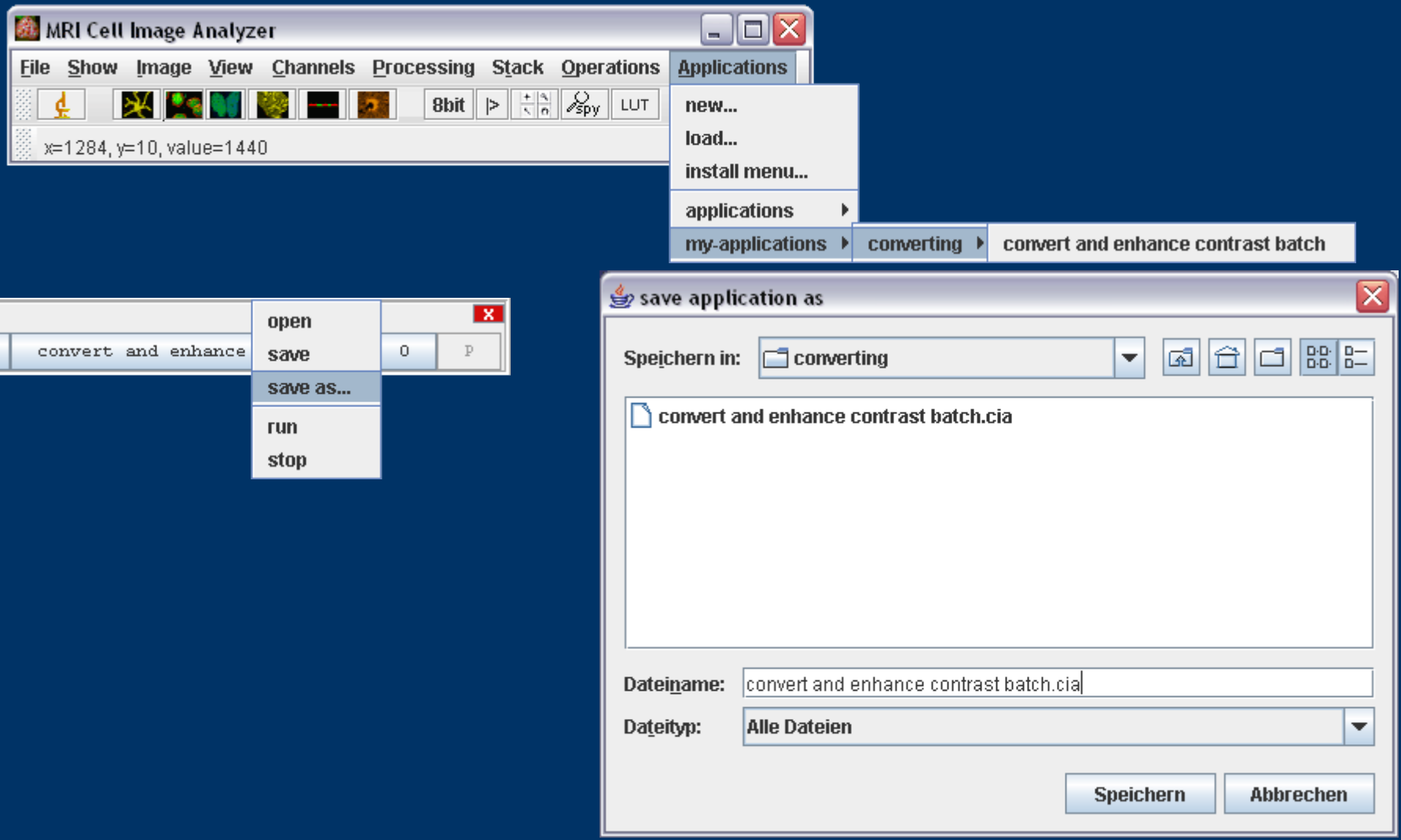
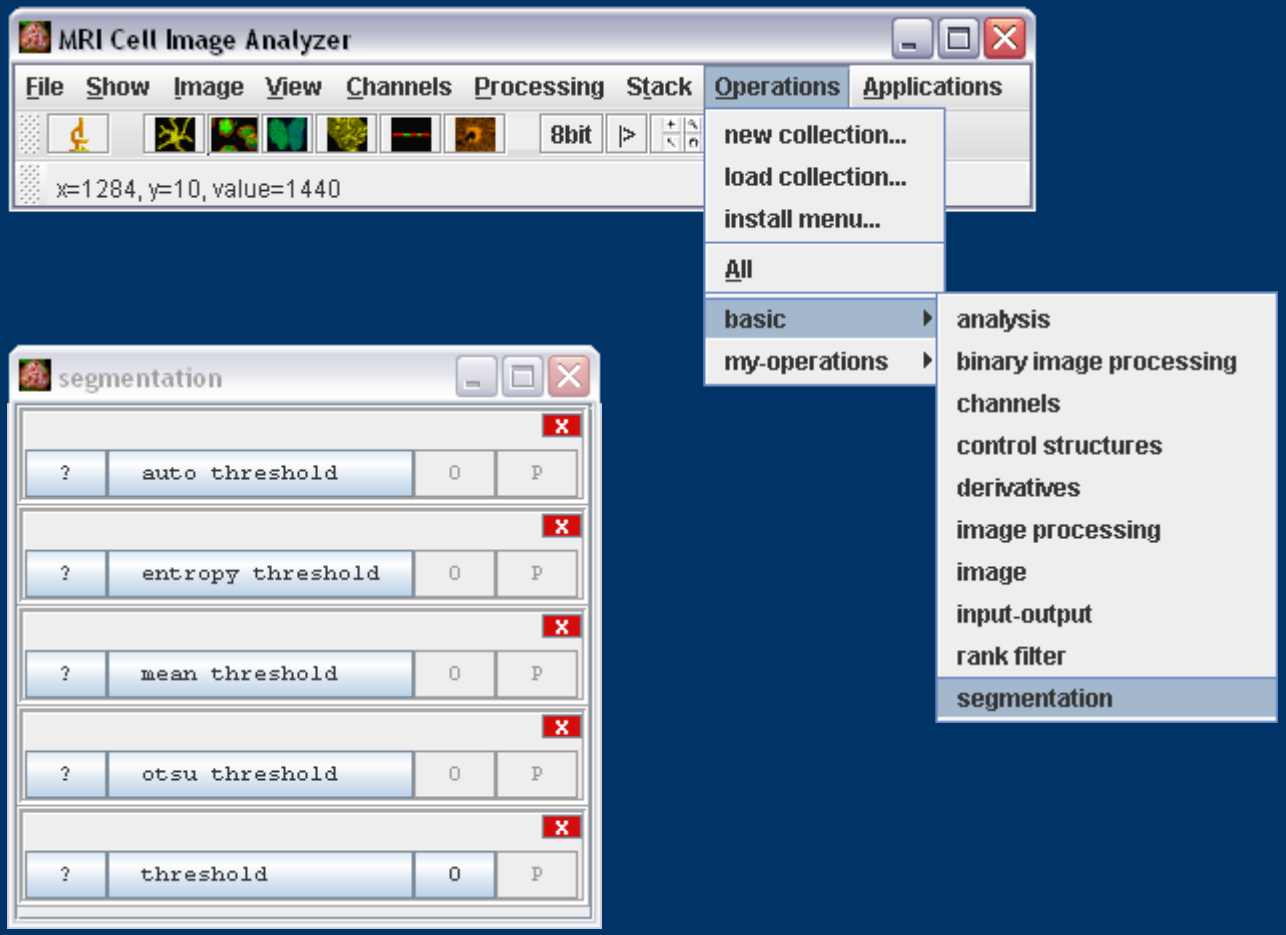
MRI Cell Image Analyzer - application prototyping framework

- build applications by connecting operations



MRI Cell Image Analyzer - application prototyping framework

- build applications by connecting operations



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interactive tools

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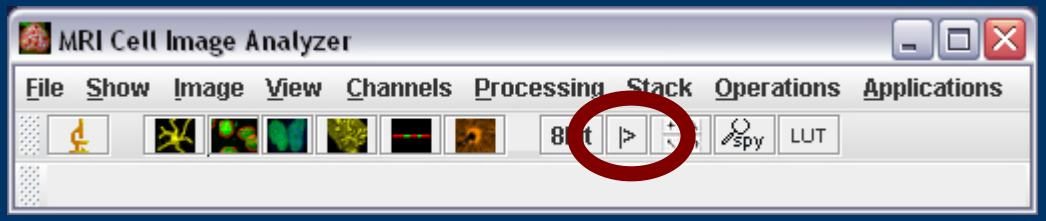
MRI Cell Image Analyzer - interactive tools

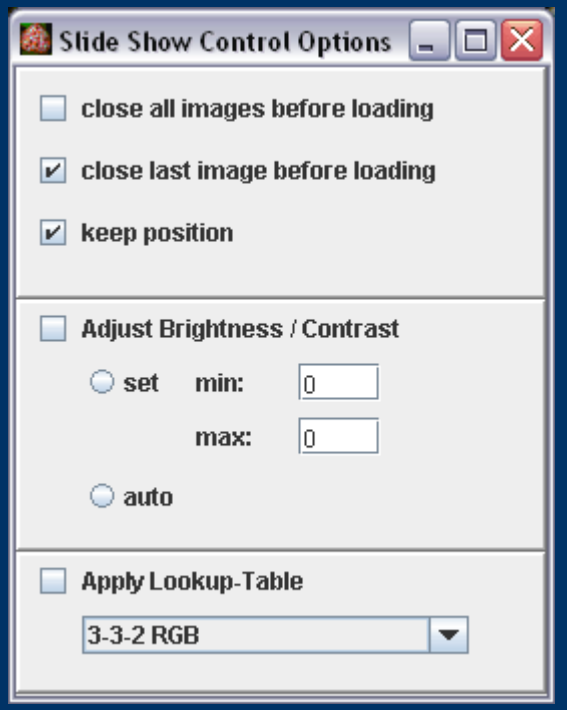
- interactive tools
  - for experimenting
  - semi-automatic solutions
- basic tools
  - slide show control
  - lookup tables
  - brighness and contrast adjuster / threshold adjuster (imagej)
  - tool box (zoom, select, measure, calibrate, annotate)
  - pixel spy
  - image calculator (imagej)
  - channel chooser, channel mixer
  - merge and split channels (imagej)

MRI Cell Image Analyzer - interactive tools – slide show control



- open images in a folder one after the other
- ^ select folder
- |< to first image
- < one image back
- > one image forward
- >| to last image
- § reload current image

options



- keeps zoom
- keeps window position

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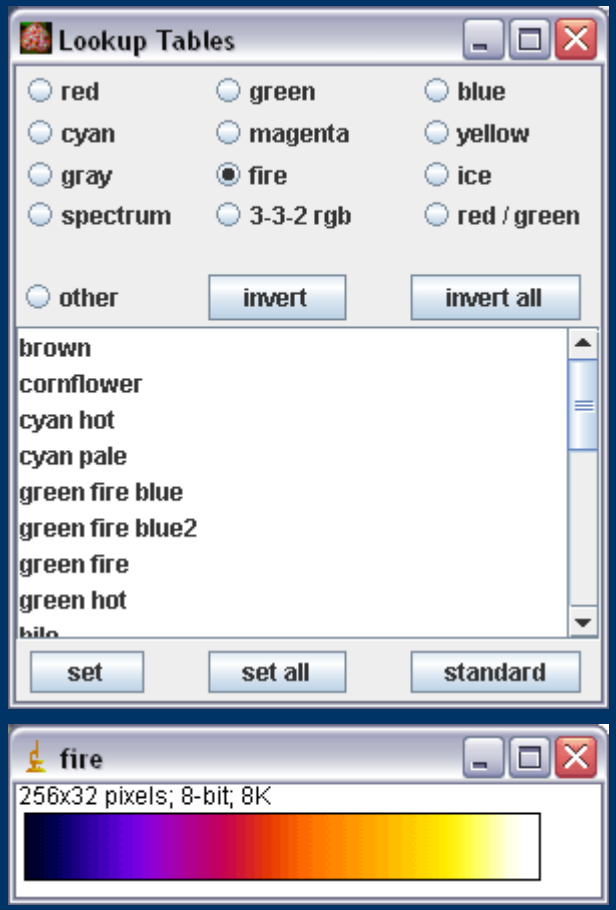
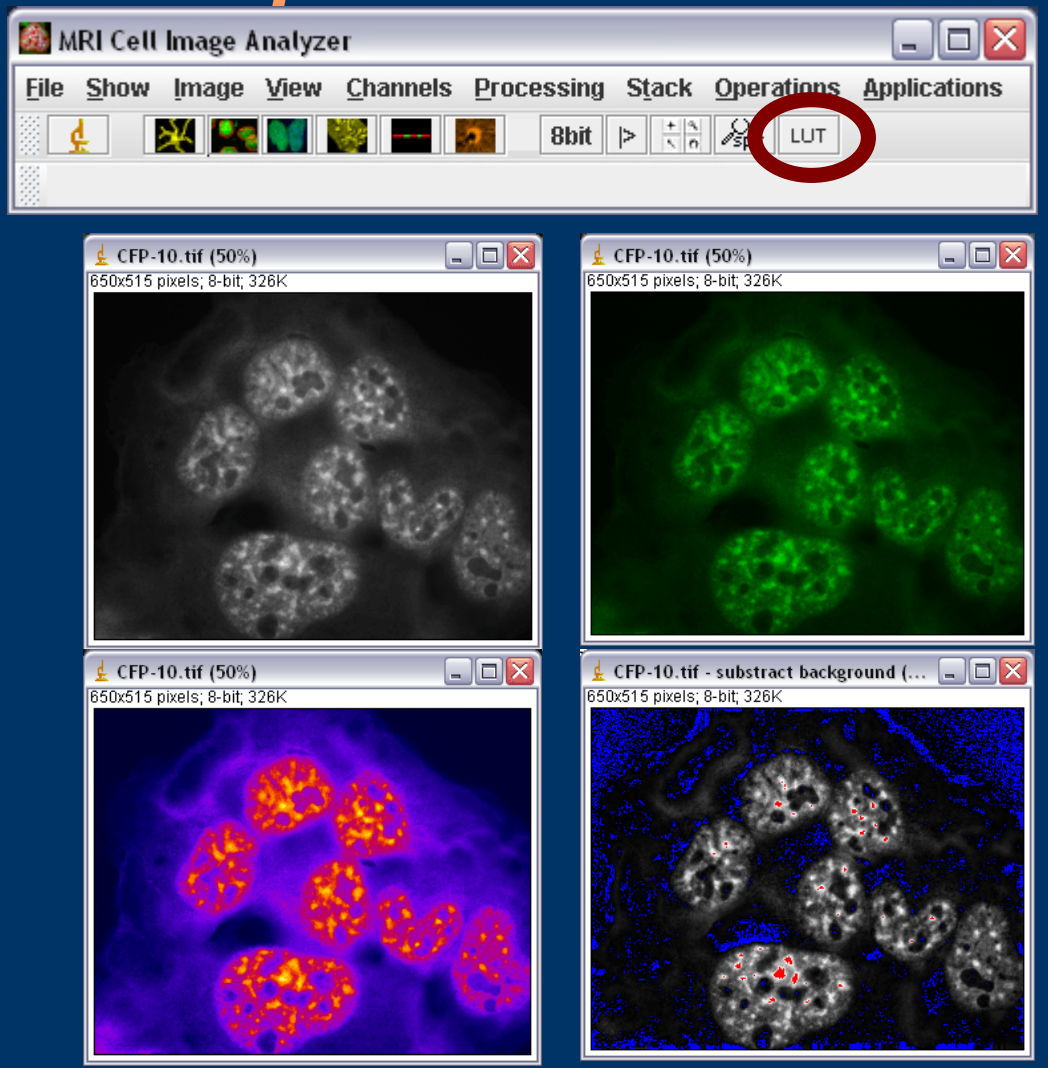
MRI Cell Image Analyzer - interactive tools – lookup tables tool





- Each intensity value 0-255 is interpreted as one color
- Lookup table defines the mapping

- use hilo to see adjust brightness
  - 0 is displayed blue
  - 255 is displayed red
  - 1-254 greyscale

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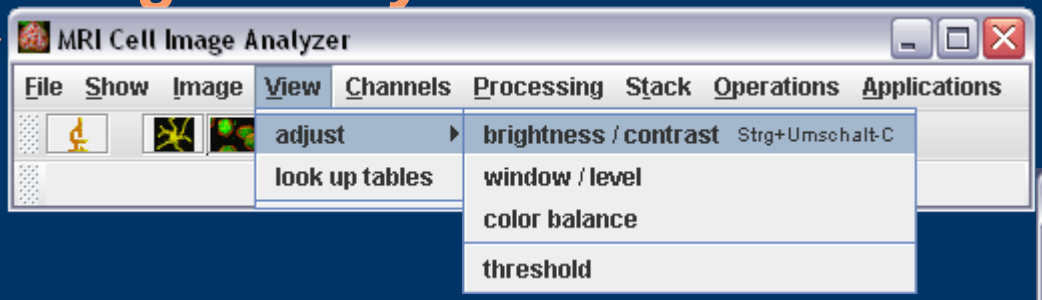
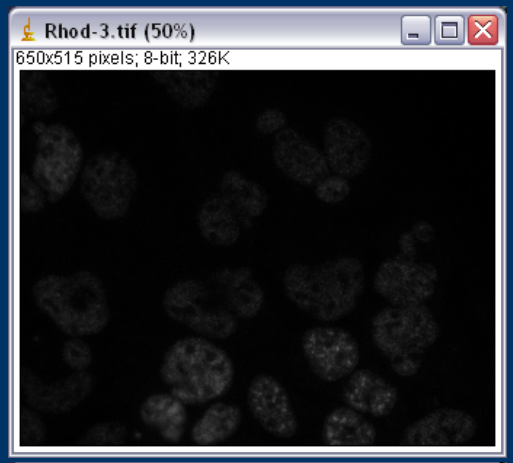
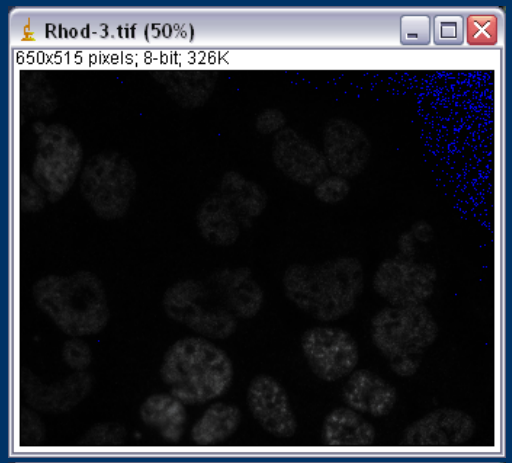
MRI Cell Image Analyzer - interactive tools – b&c adjuster (imagej)

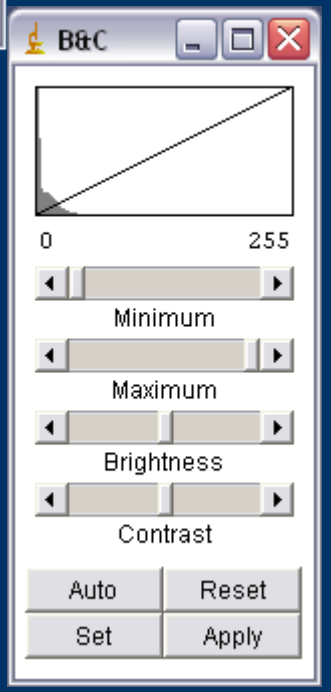


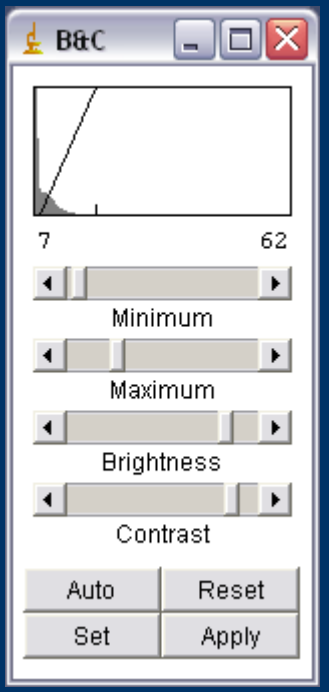
original

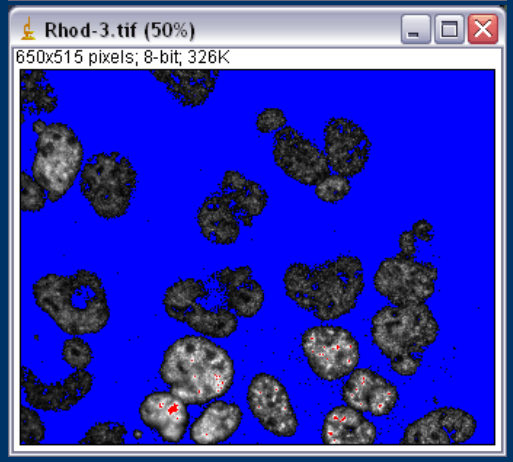


original with hilo lut

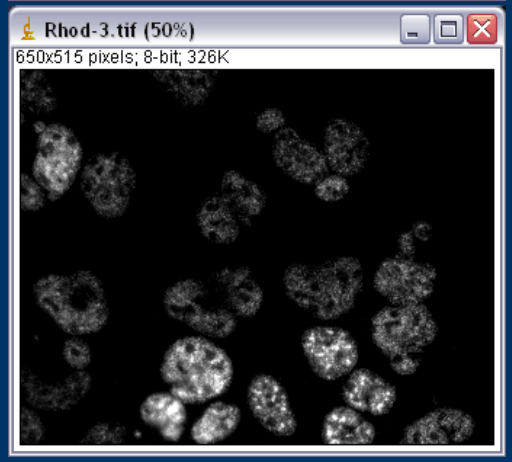




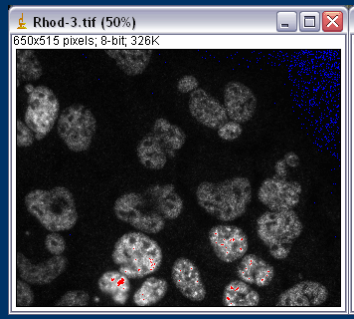




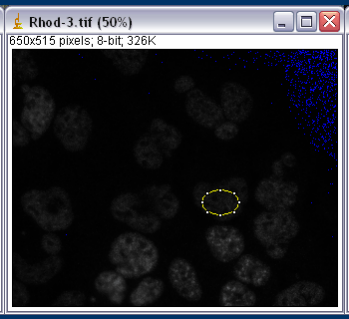
adjusted with hilo lut



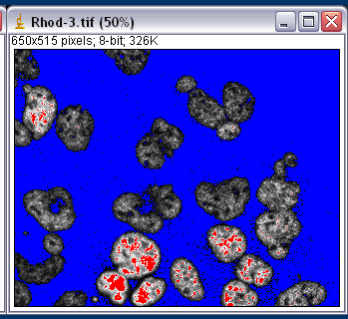
adjusted



auto adjusted based on whole image



selection



auto adjusted based on selection

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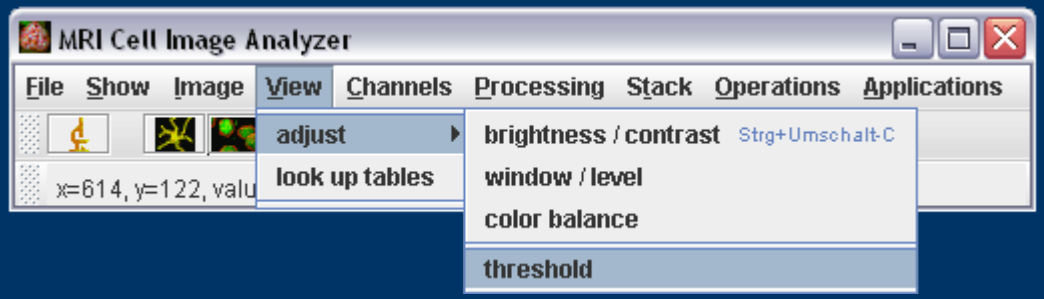
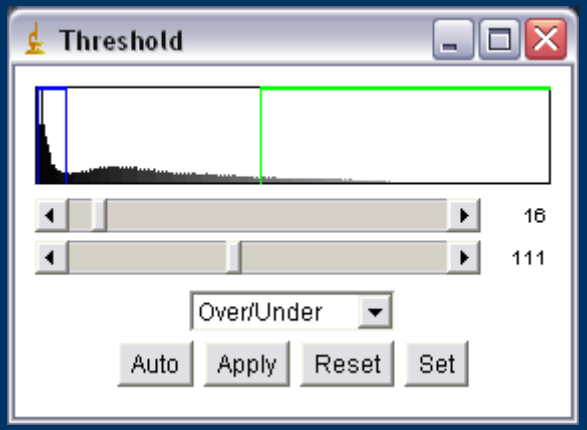
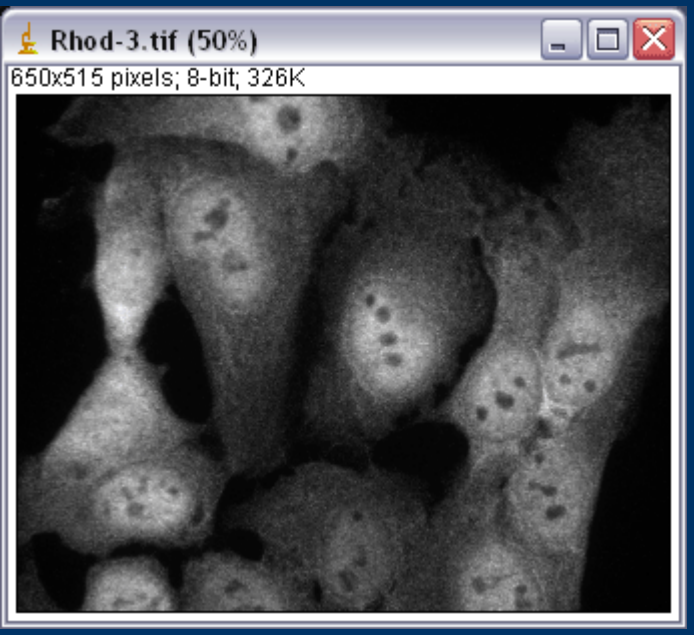
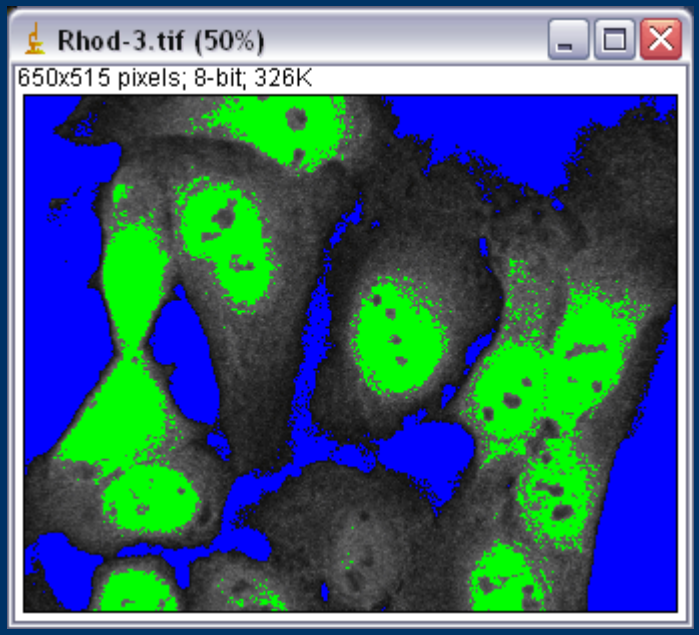
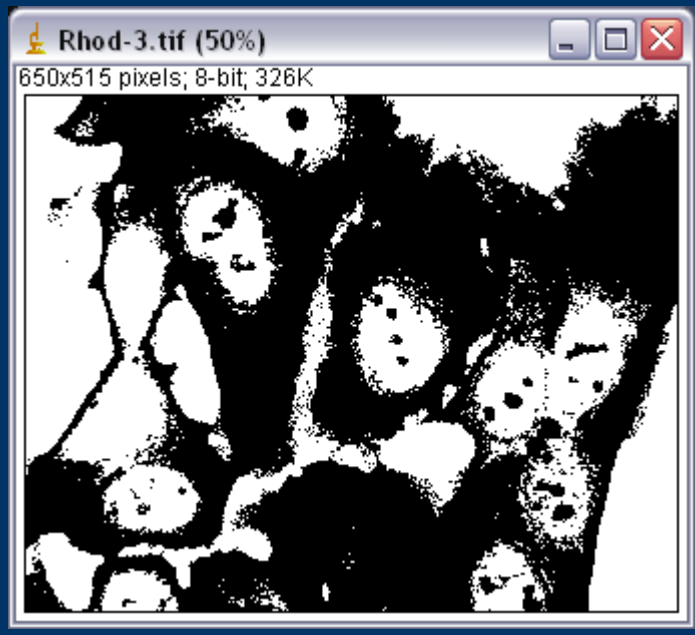
MRI Cell Image Analyzer - interactive tools – threshold adjuster (imagej)











- create a mask
  - image with values 0 and 255

- Set all pixels
  - Below min and above max to 255
  - Between min and max to 0

Create selection from mask or use image arithmetic to define / exclude regions in the original image

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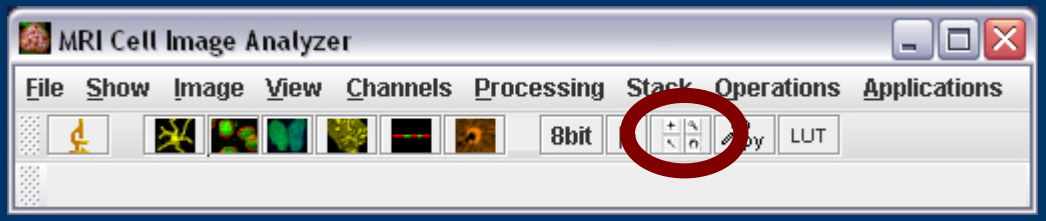
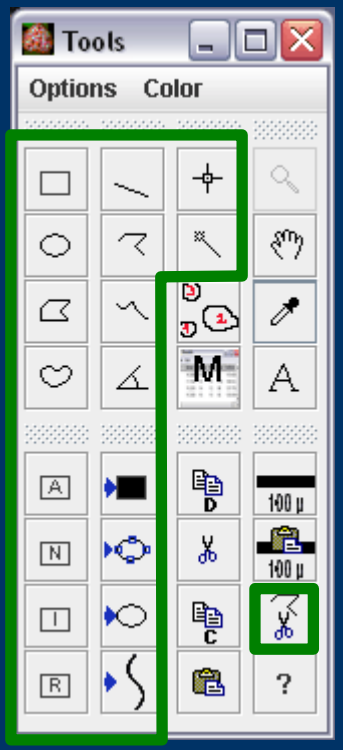
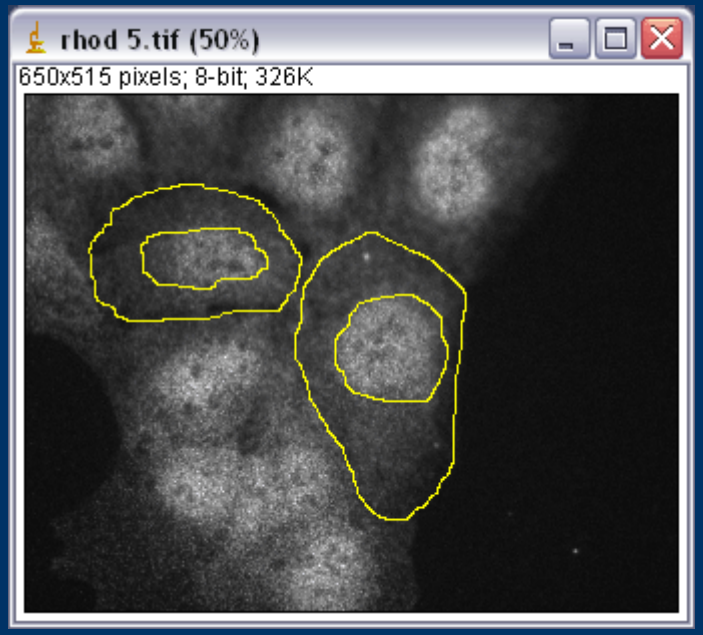
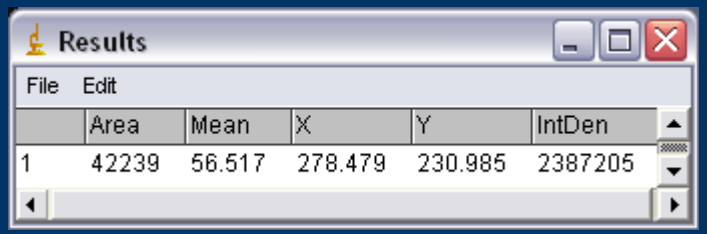
MRI Cell Image Analyzer - interactive tools - toolbox





- to restore a selection or to transfer it between images use
- to delete the last segment of a polygon selection use
- use right-click to finish polygon selections
- 2d selections will automatically create the last segment to close the selection
- to create complicated selections use
  - Shift to add
  - Alt to subtract





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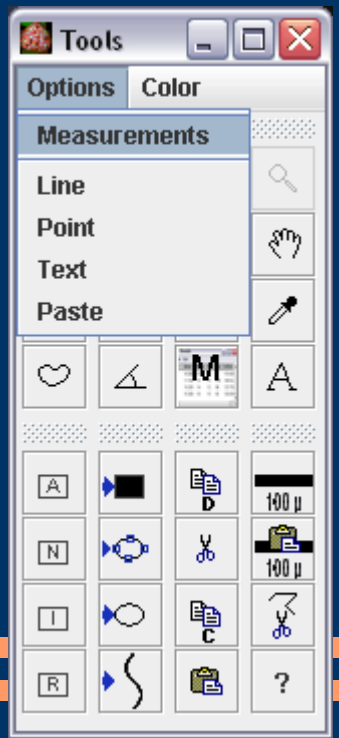
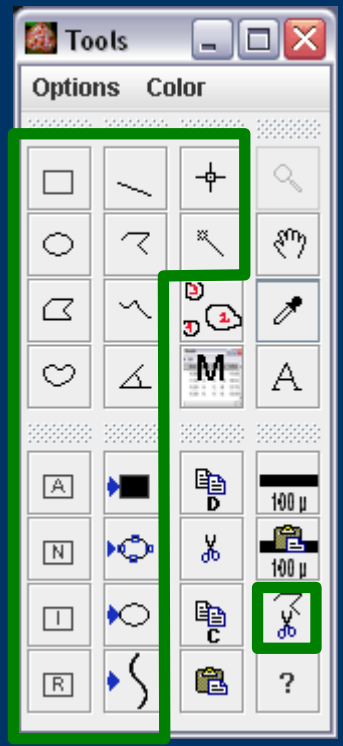
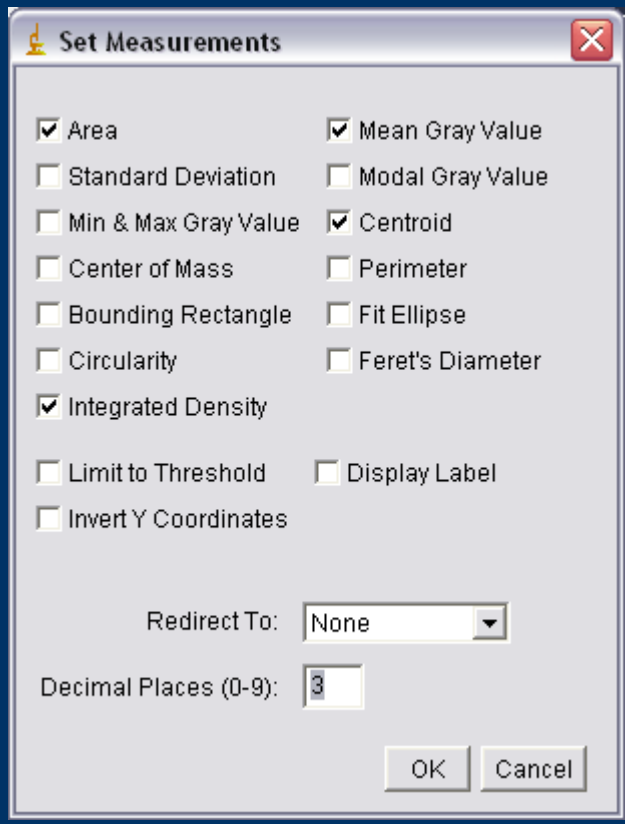
MRI Cell Image Analyzer - interactive tools - toolbox

- 2d selection to measure
  - areas
  - intensities
  - form features
  - coordinates
- 1d selections to measure
  - lengths
  - intensities
  - angles
  - coordinates
- 0d (point) selections to
  - count
  - measure intensities
  - measure coordinates

- use options>measurements to tell what you want to measure
- use redirect to make a selection in one image (usually a mask) and measure in an other
- use CTRL-M or to measure a selection







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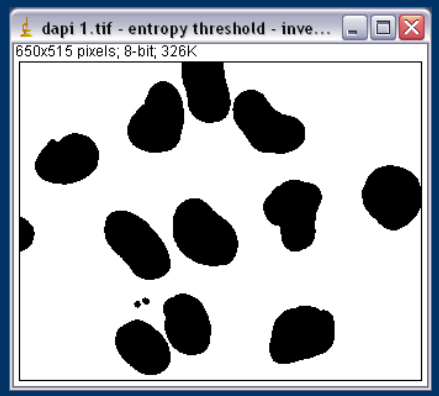
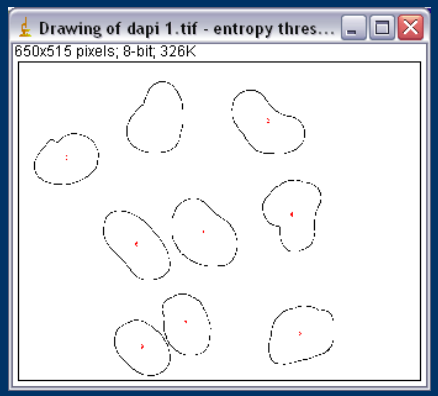
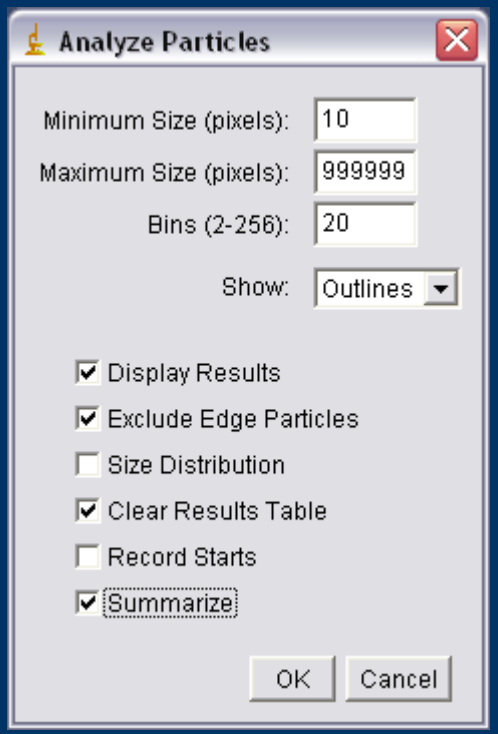
MRI Cell Image Analyzer - interactive tools - toolbox

use to find and measure all objects defined by a mask



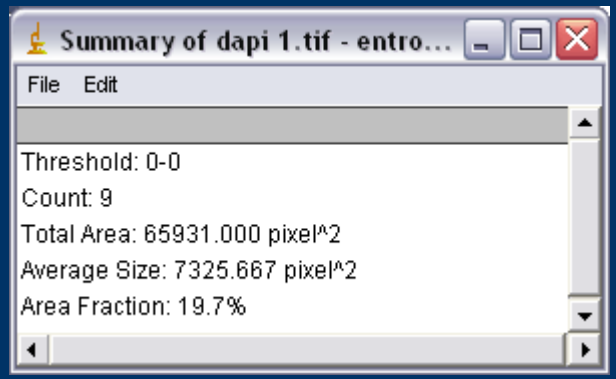




Results

File Edit

	Area	Mean	X	Y	IntDen
1	7740	149.797	224.502	93.851	1159430
2	7869	142.506	400.649	100.973	1121381
3	6483	129.911	77.919	157.903	842214
4	7669	136.592	445.337	243.570	1047527
5	8563	106.800	298.611	278.465	914531
6	7790	109.767	190.786	296.082	855088
7	6086	93.757	271.349	424.901	570608
8	7886	123.009	456.981	441.215	970051
9	5845	95.390	200.192	462.430	557552



don't forget to redirect to the original image if you want to measure intensities

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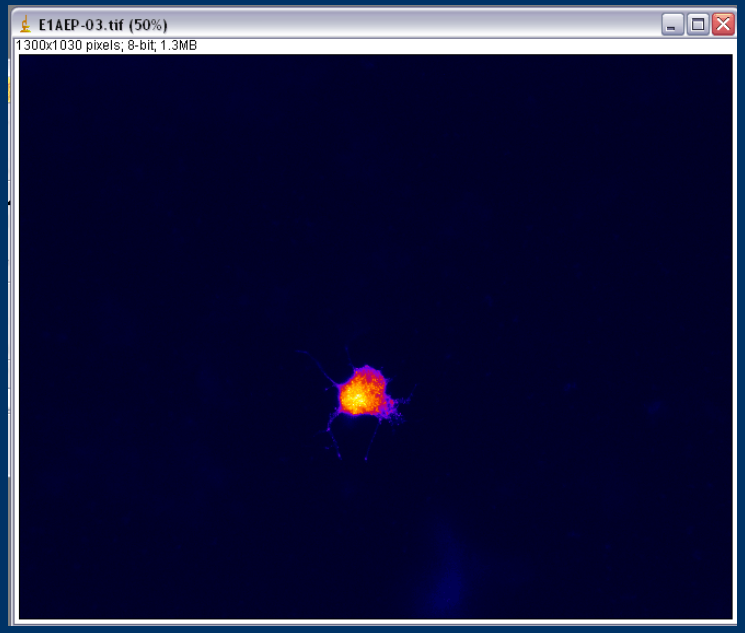
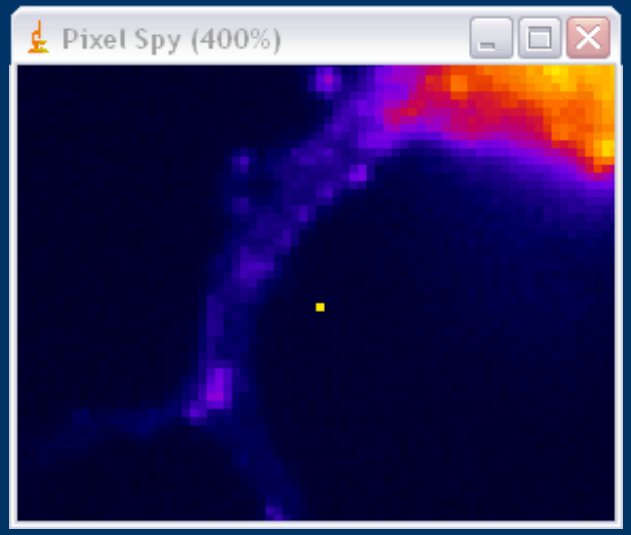
MRI Cell Image Analyzer - interactive tools – pixel spy

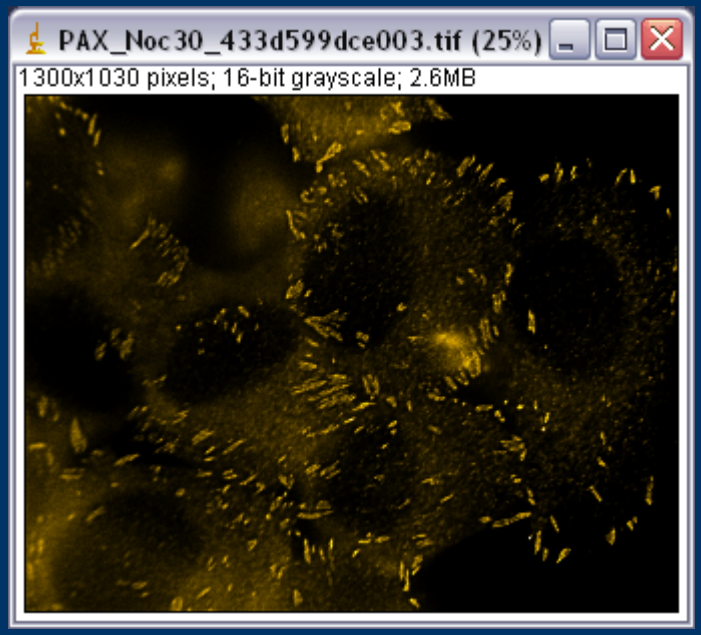


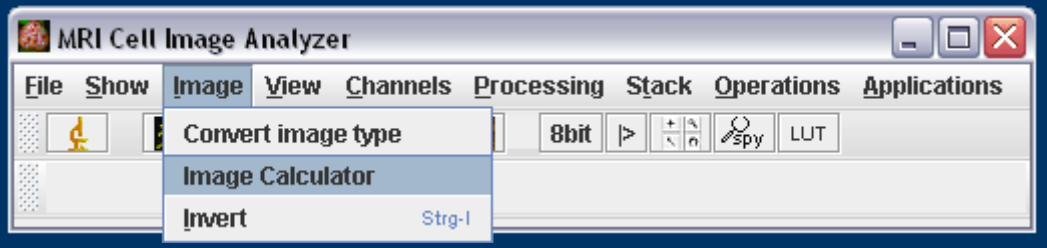


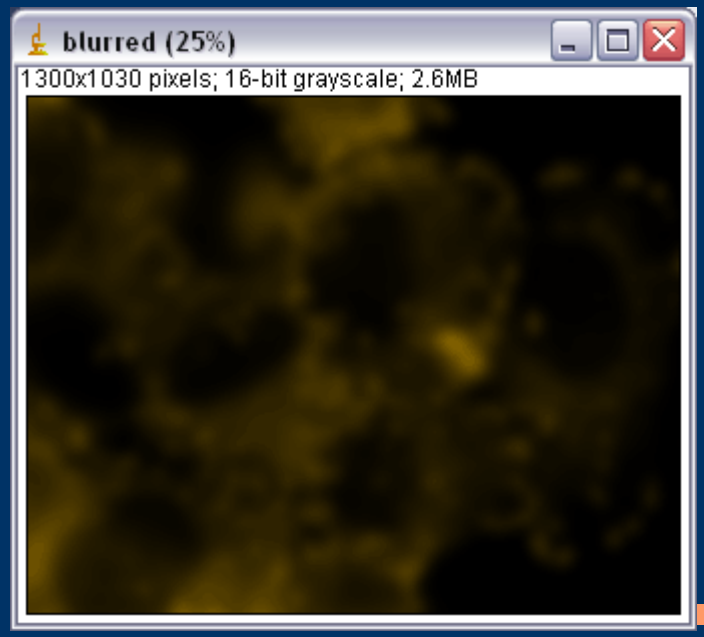
a magnifying glass that shows the region under the mouse pointer

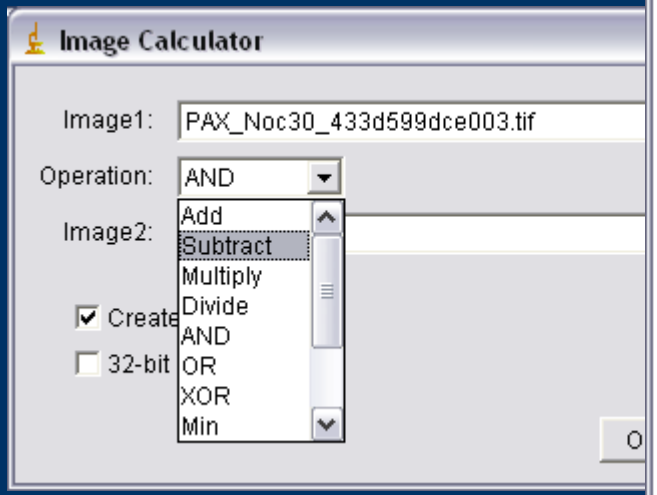
- you can change the zoom and window size of the pixel spy

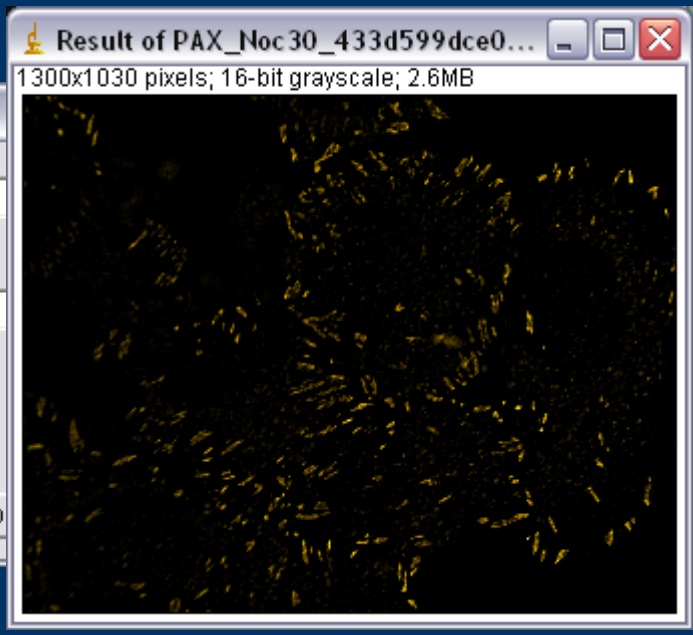
MRI Cell Image Analyzer - interactive tools – image calculator (imagej)











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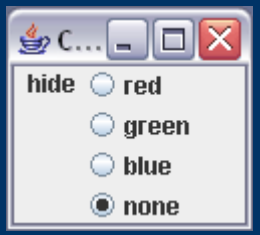
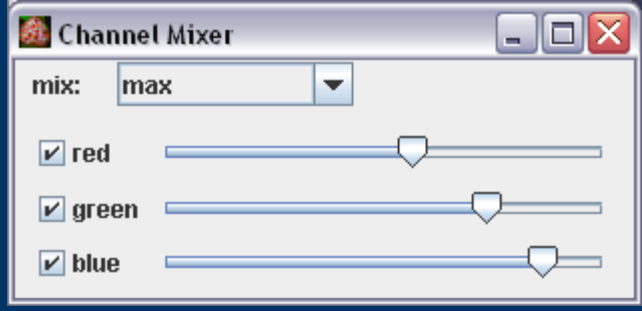
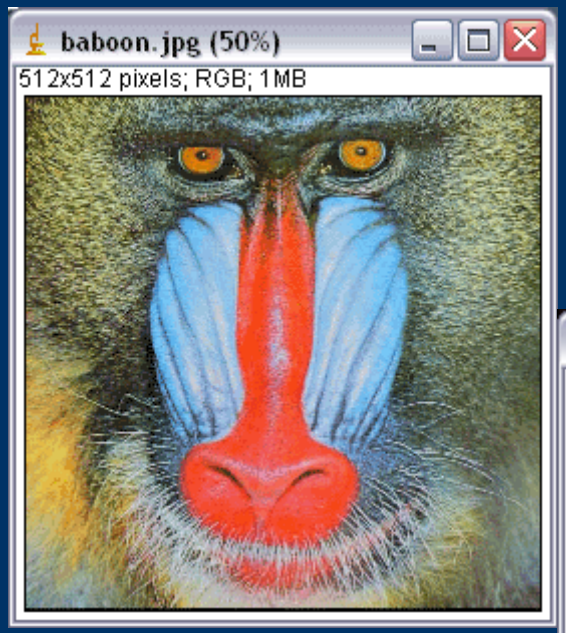
MRI Cell Image Analyzer - interactive tools – channel mixer

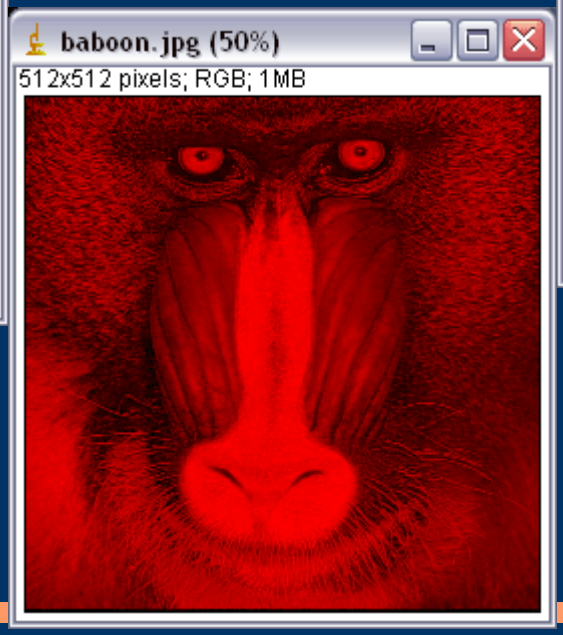


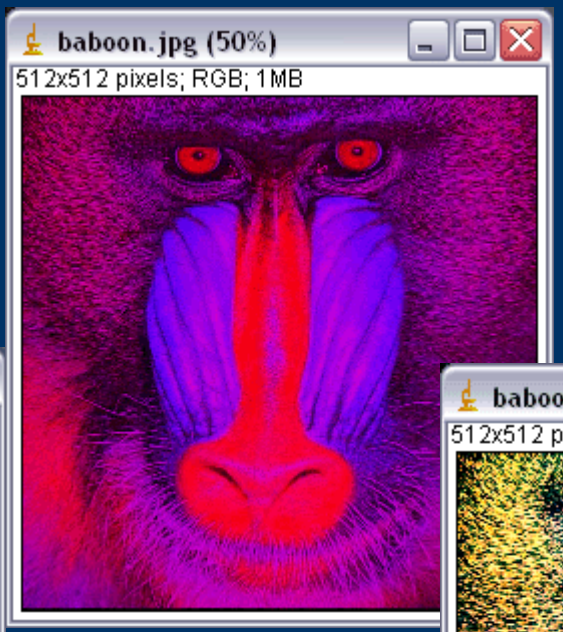




original

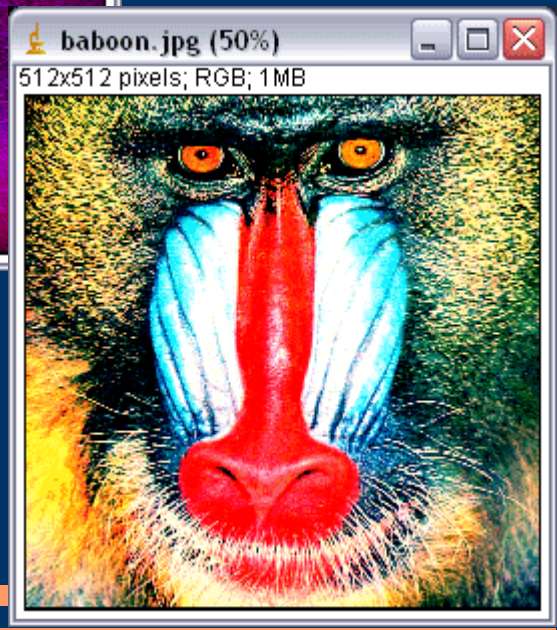
red channel only





red and blue

Adjusted min and max of all colors



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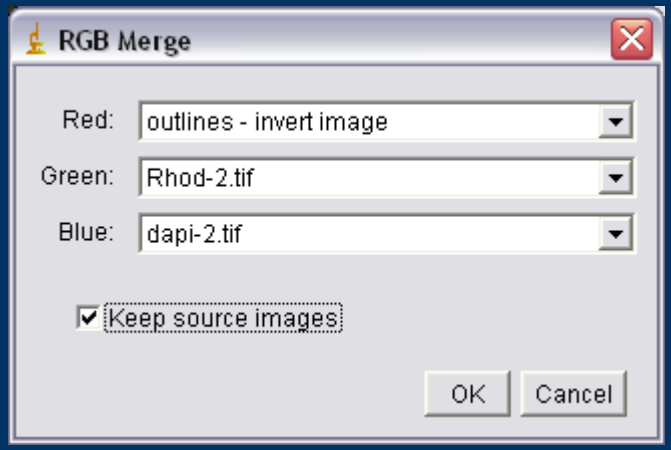
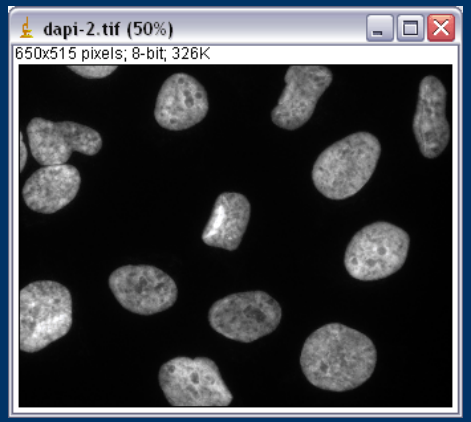
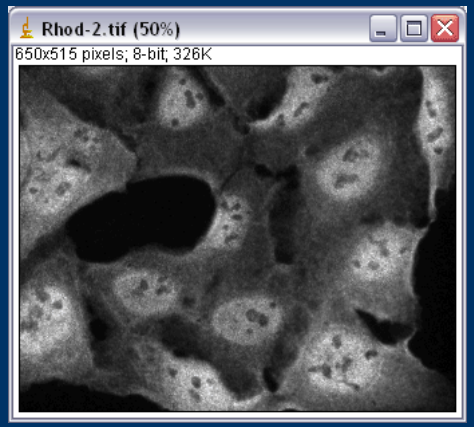
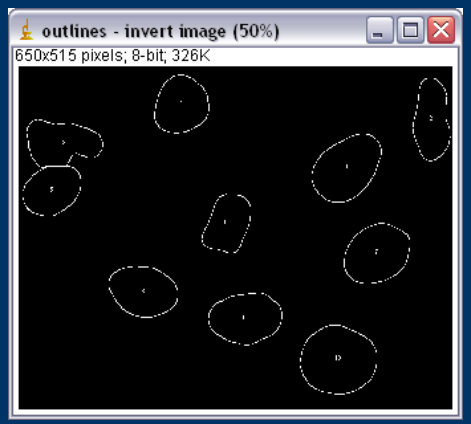
MRI Cell Image Analyzer - interactive tools – merge channels (imagej)









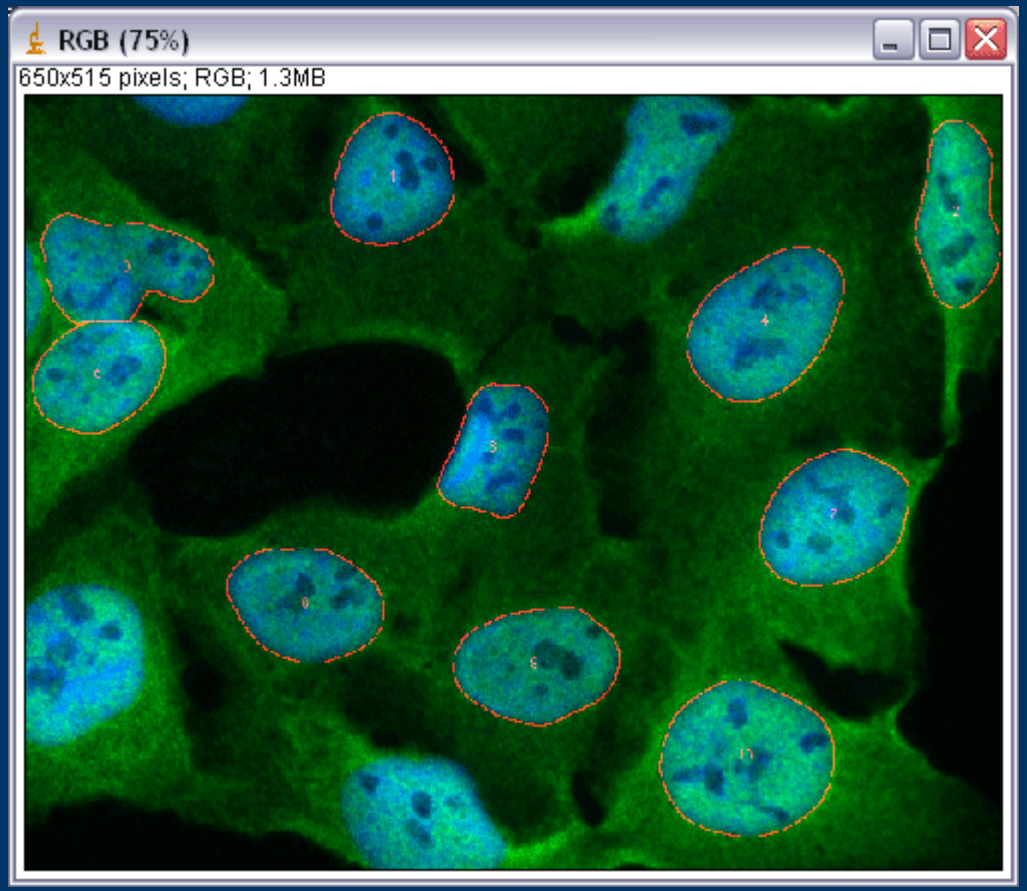


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image processing and analysis

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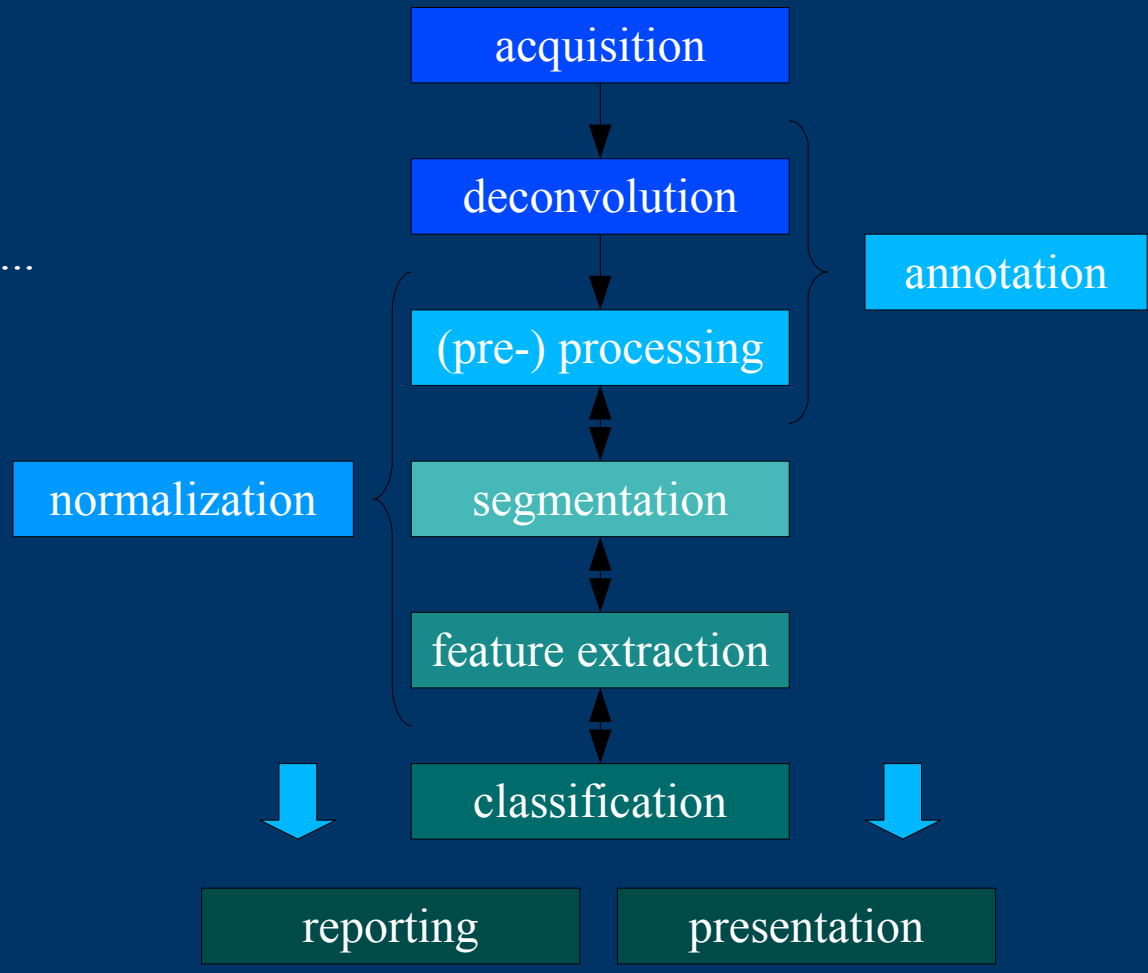
MRI Cell Image Analyzer – image processing and analysis

- processing
  - image > image
    - filter – sharpen, blur, subtract background, ...
- segmentation
  - image > mask
    - mask - image with 2 intensity values
    - seperate objects from background and objects from each other
    - threshold, watershed, dilate, erode
- feature extraction
  - image > feature vector (numbers)
    - lengths, areas, intensities, moments, ...
- classification
  - feature vector > classes normal cells, apoptotic cells, ...



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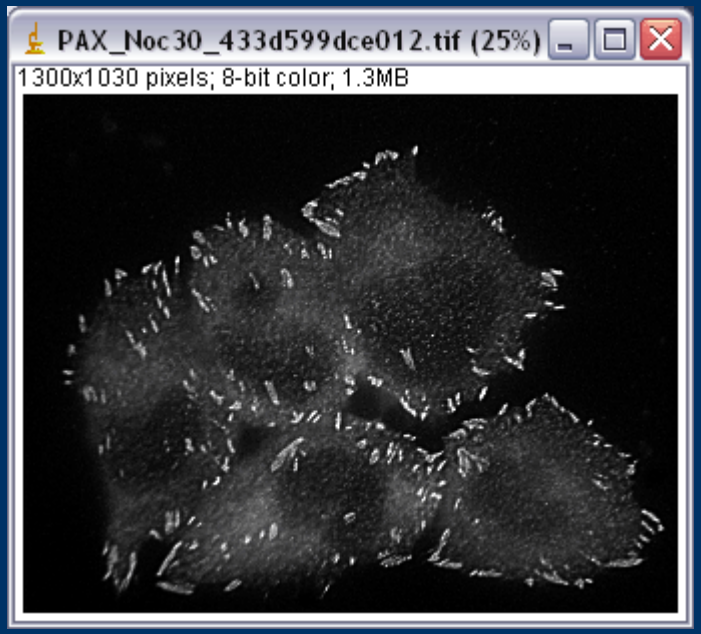
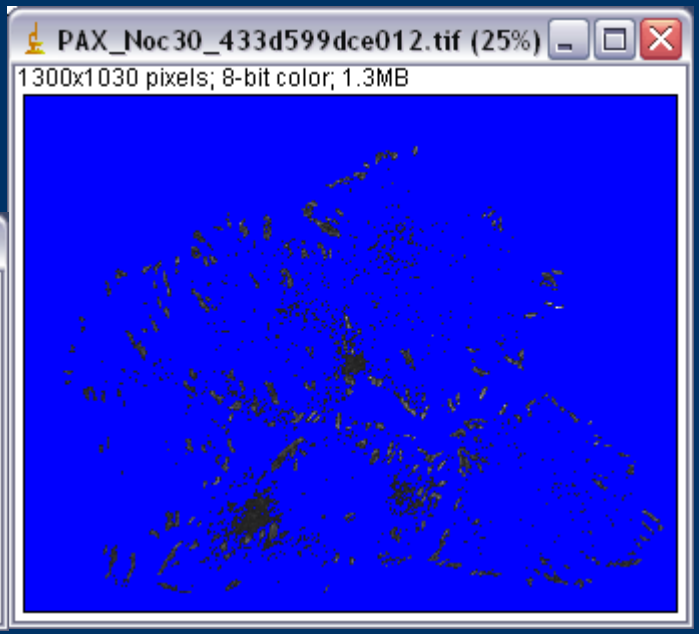
MRI Cell Image Analyzer - image analysis - example



- number and average size of plaques
  - compare between experiment and control
- simple approach:
  - threshold between intensities min and max
  - find objects between min and max size
  - measure

Doesn't work because of same grey levels in plaques and background



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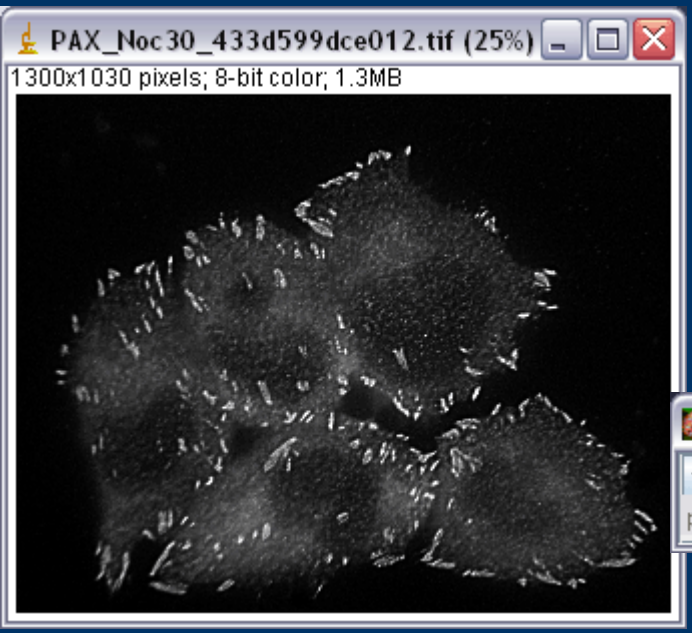
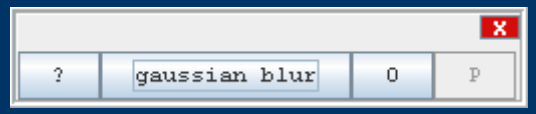
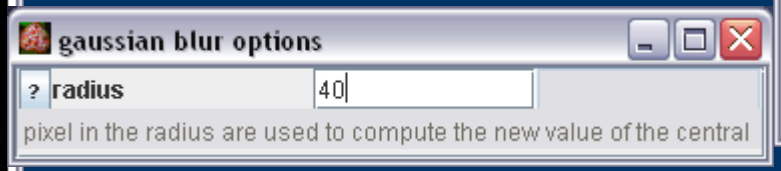
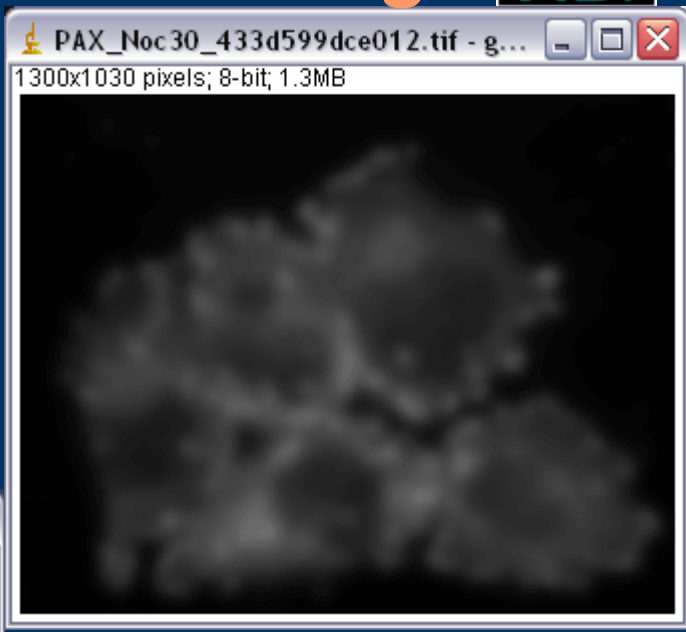
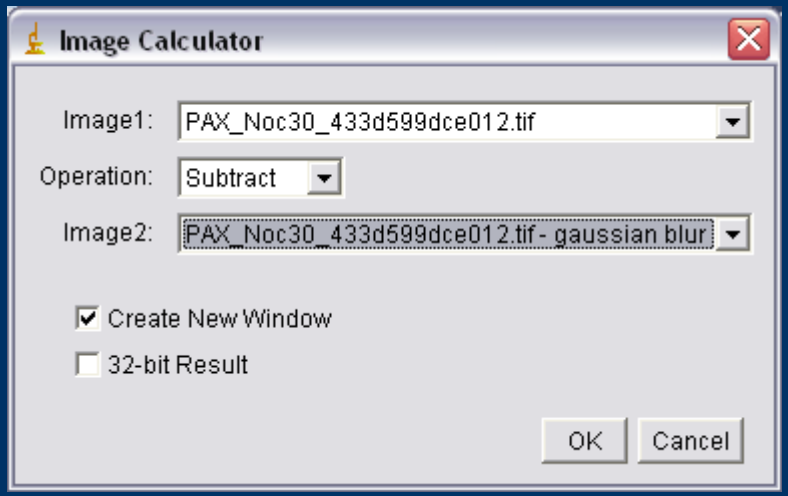
MRI Cell Image Analyzer - image analysis – example - preprocessing











subtract a blurred version of the image from the original image

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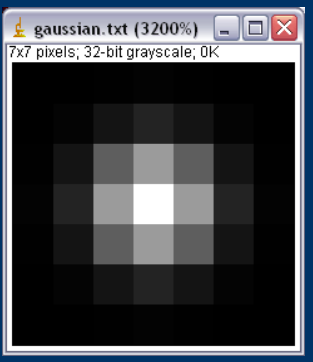
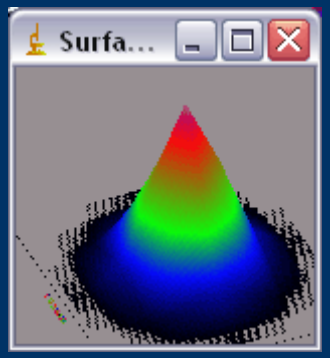
MRI Cell Image Analyzer – image gaussian blur





0	0	1	2	1	0	0
0	3	13	22	13	3	0
1	13	59	97	59	13	1
2	22	97	159	97	22	2
1	13	59	97	59	13	1
0	3	13	22	13	3	0
0	0	1	2	1	0	0

gaussian kernel

normal or gaussian distribution

$$G(x,y)=\frac{1}{2\cdot\pi\cdot\sigma^2}\cdot e^{\frac{-x^2+y^2}{2\cdot\sigma^2}}$$

convolve image with normalized gaussian kernel

new value of pixel

weighted sum of pixels in neighborhood
weighted with the values of the kernel

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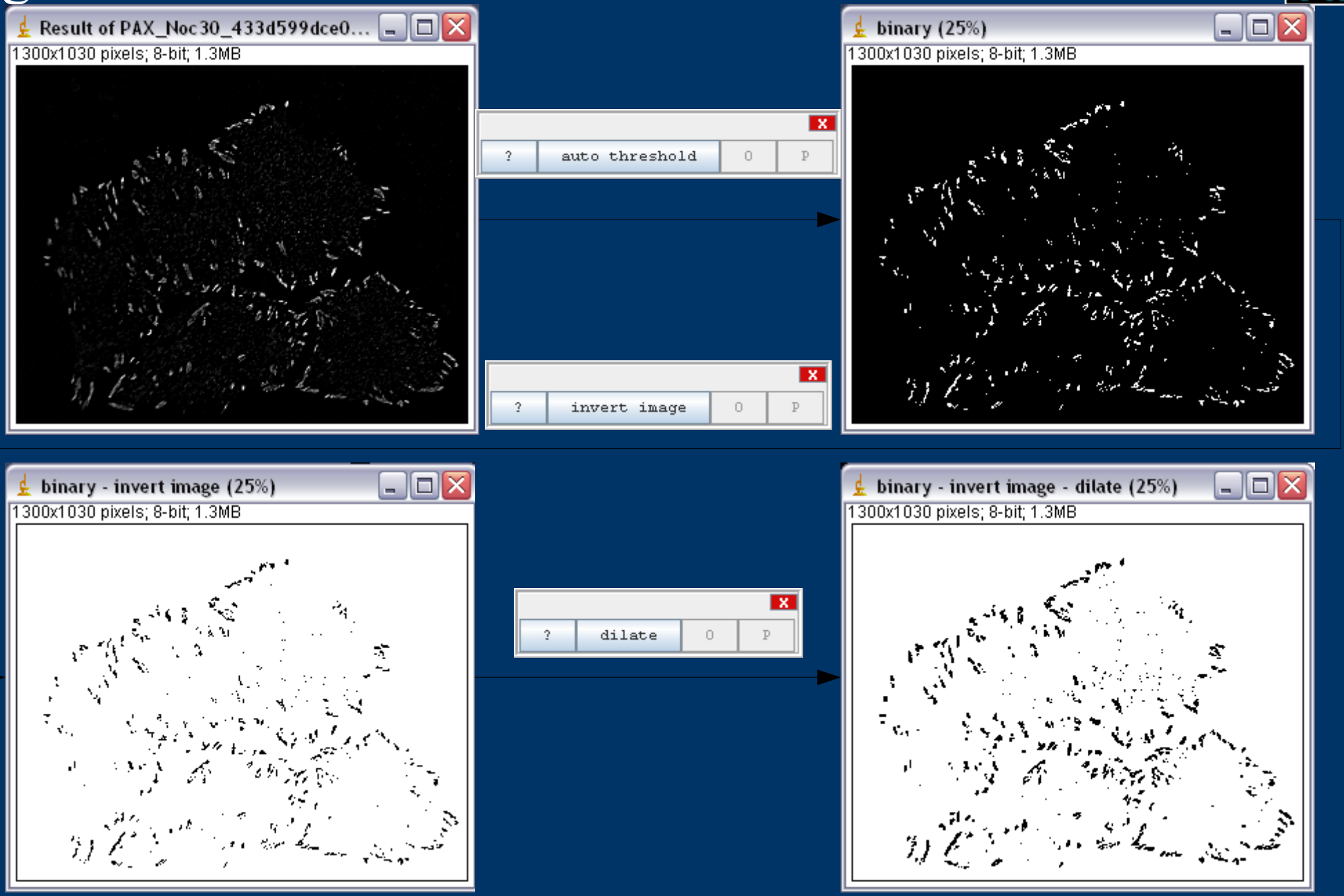
MRI Cell Image Analyzer – image analysis - example - segmentation



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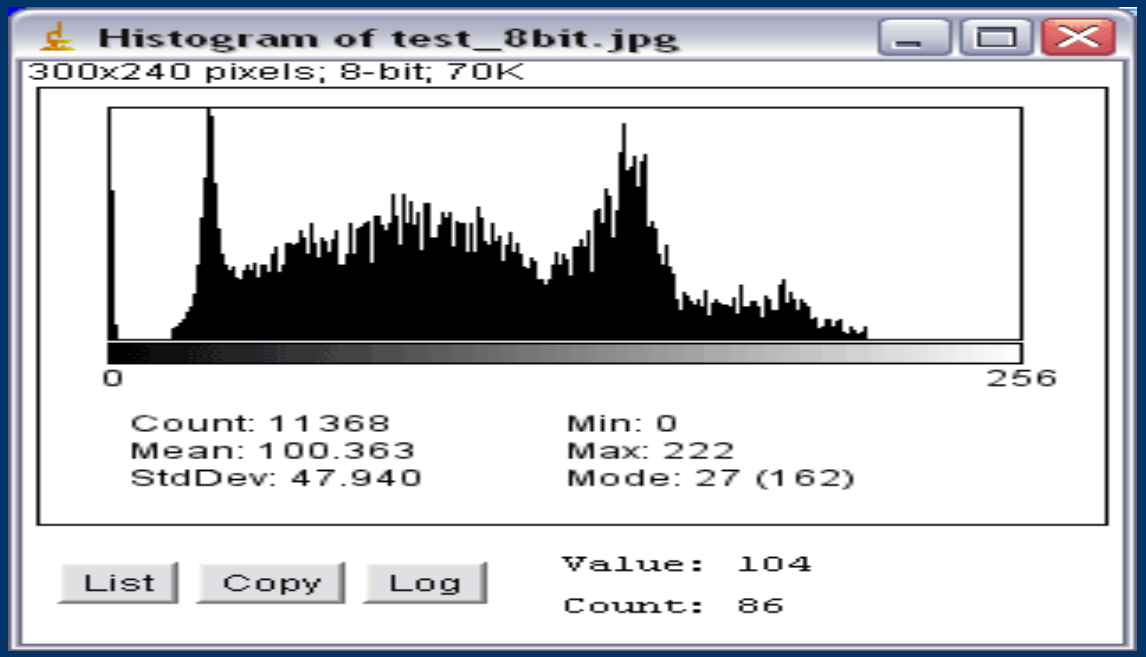
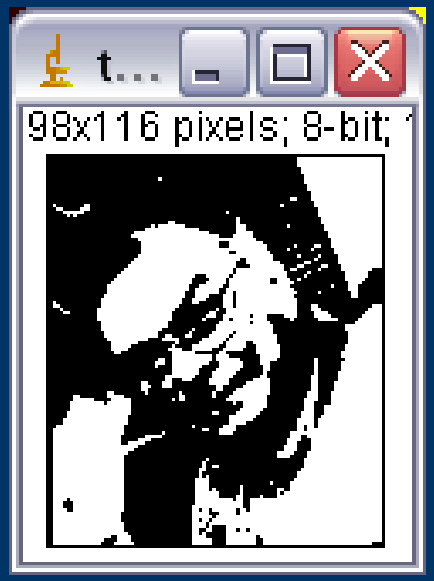
MRI Cell Image Analyzer – image auto threshold





Histogram:

x: greylevel

y: frequency (count of pixels with greylevel x in the image)

find greylevel that divides the histogram so that:

$$threshold = \frac{average\ background + average\ objects}{2}$$

$$t = \frac{\frac{\sum_{i=0..t} h(i) \cdot i}{\sum_{i=0..t} h(i)} + \frac{\sum_{i=t..255} h(i) \cdot i}{\sum_{i=t..255} h(i)}}{2}$$

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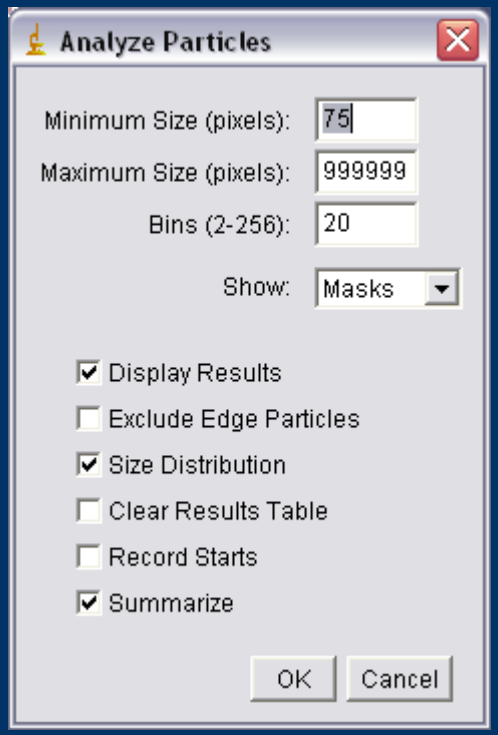
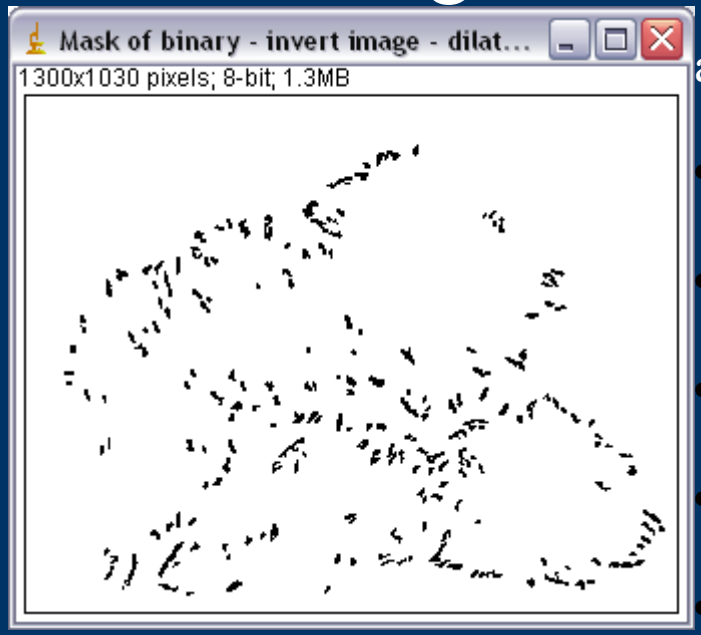
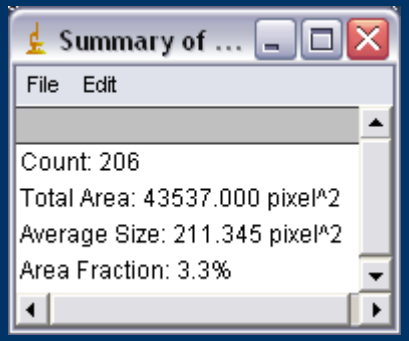
MRI Cell Image Analyzer – image analysis feature extraction / measuring

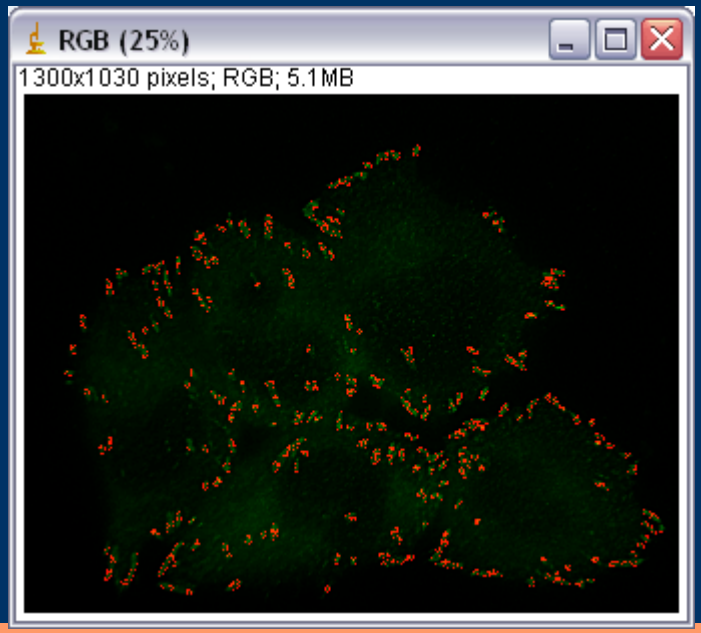




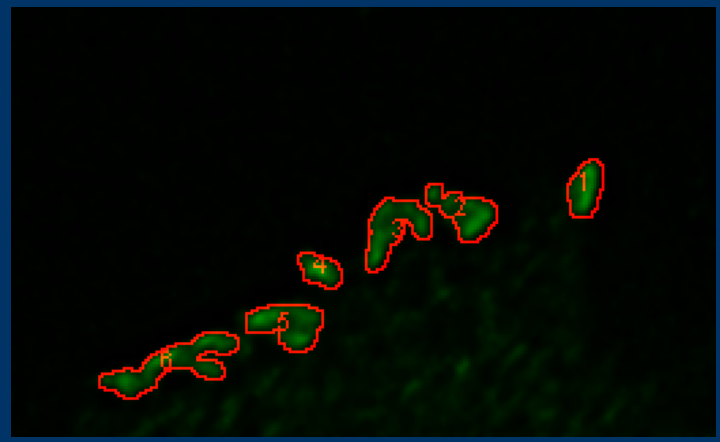
analyze particles / find objects operation

- only particles bigger than 75 square pixel
- gives the number of found objects: 206
- gives the average size in square pixel 211
- draws a mask of found objects
- draws outlines of found objects





- merge of outlines and original image to check quality of result



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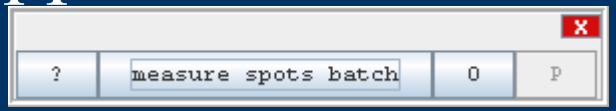
applications

MRI Cell Image Analyzer – image analysis applications – 1. measure plaques



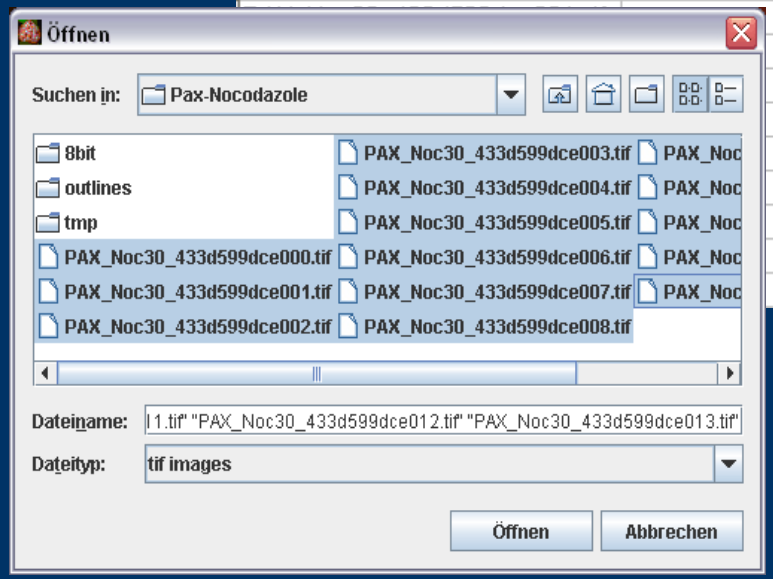


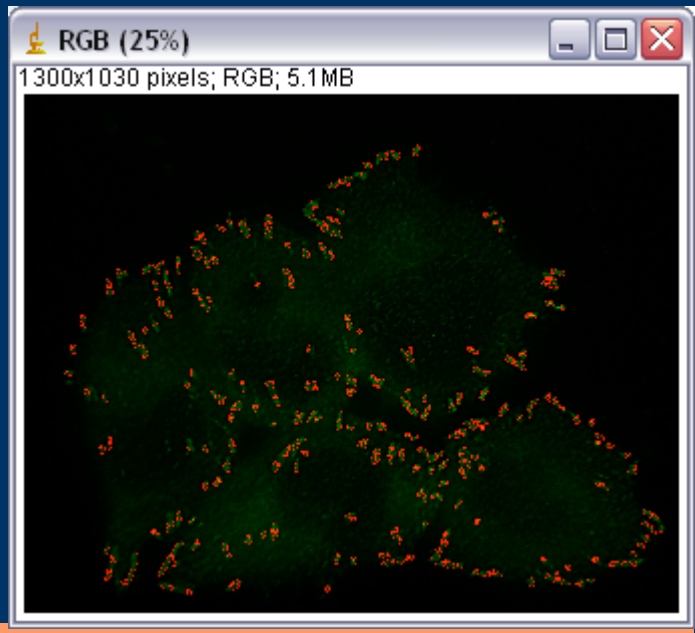
image	number of objects	Area	folder
PAX_Noc30_433d599dce000.tif	216	215,32	Z:\baecker\ory\Pax-Nocodazole\
	267	175,55	Z:\baecker\ory\Pax-Nocodazole\
	267	170,35	Z:\baecker\ory\Pax-Nocodazole\
	97	201,43	Z:\baecker\ory\Pax-Nocodazole\
	165	209,04	Z:\baecker\ory\Pax-Nocodazole\
	173	160,88	Z:\baecker\ory\Pax-Nocodazole\
	108	171,12	Z:\baecker\ory\Pax-Nocodazole\
	171	168,01	Z:\baecker\ory\Pax-Nocodazole\
	60	230,27	Z:\baecker\ory\Pax-Nocodazole\
	58	208,83	Z:\baecker\ory\Pax-Nocodazole\

start by pressing
"measure spots batch"

enter all images to be analyzed

results:

- spreadsheet with measurements
- folder outlines with images of found objects



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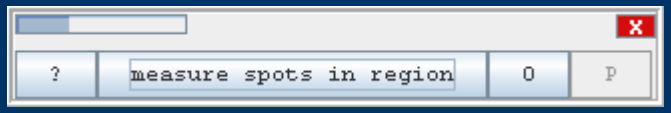
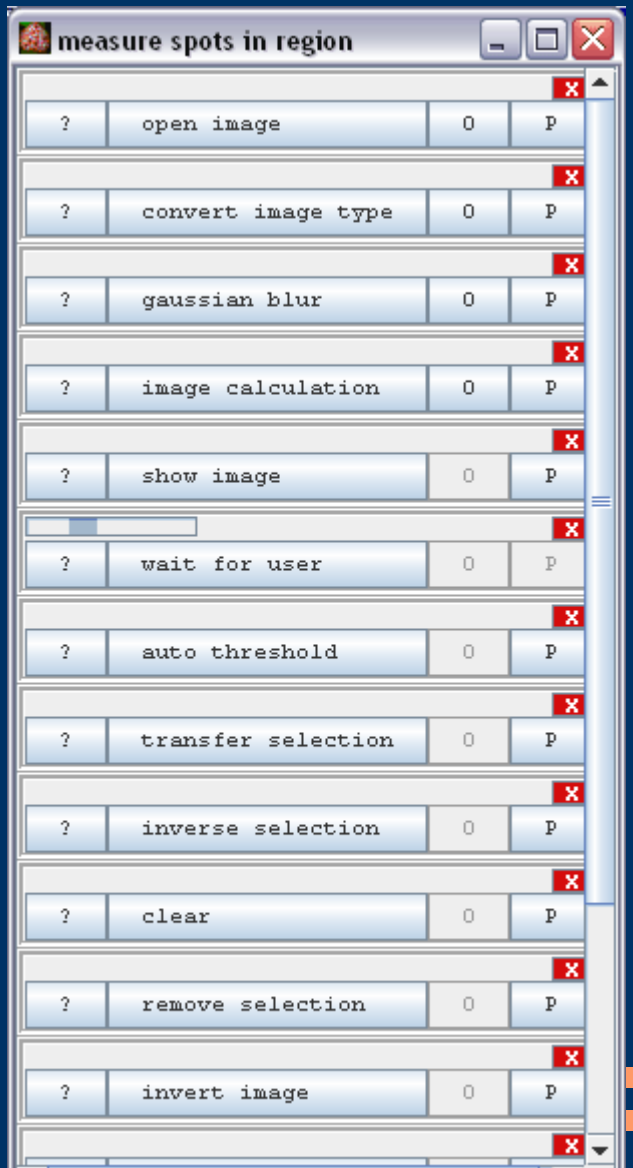
MRI Cell Image Analyzer – image analysis applications – 1. measure plaques semi-automatic

changed requirement : measure only plaquesd on some cells in the image

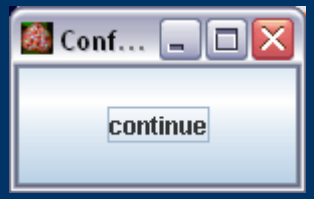


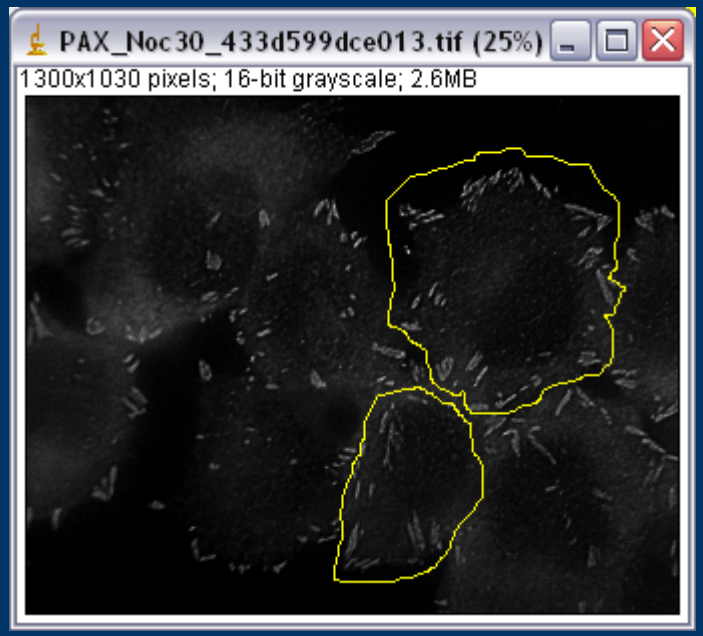


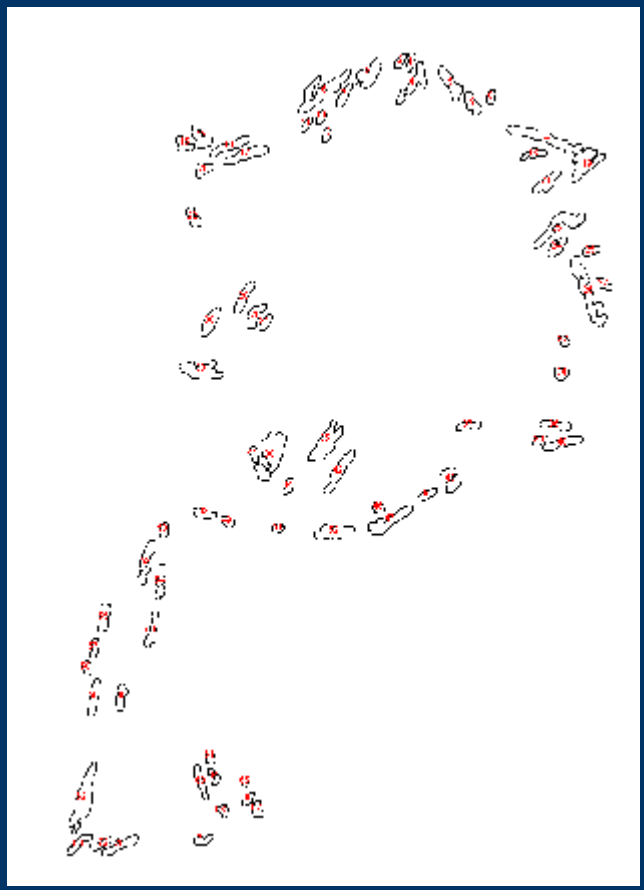
Foreach image

wait until user has marked the regions to measure

does some extra processing to avoid problems at borders of selected regions







MRI Cell Image Analyzer – applications
2. dna combing

the images:

red: combed DNA

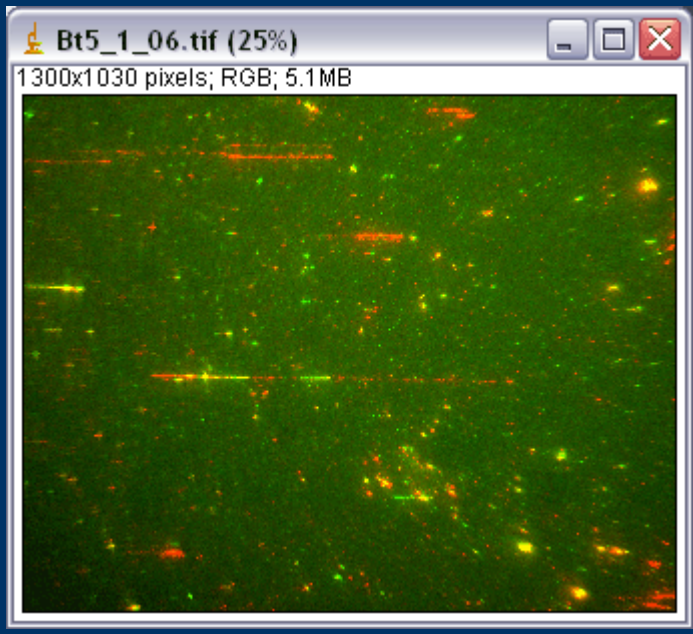
green: sites where replication takes place

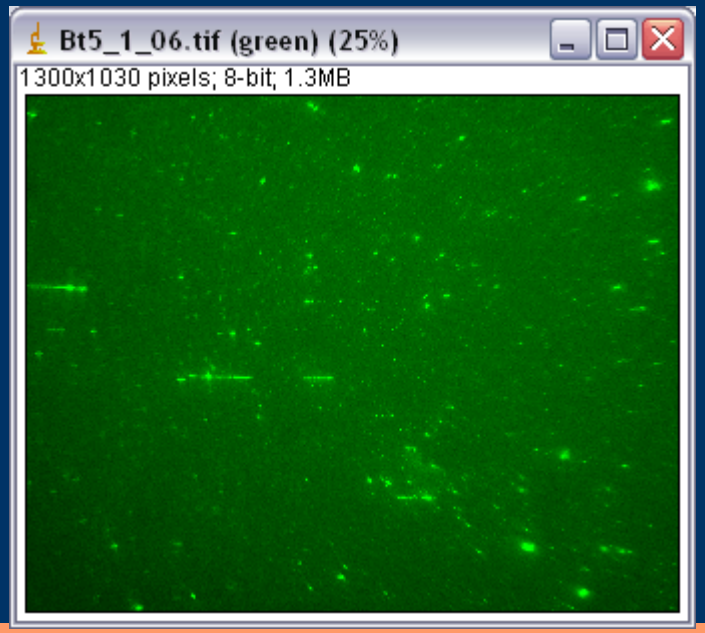
the task:

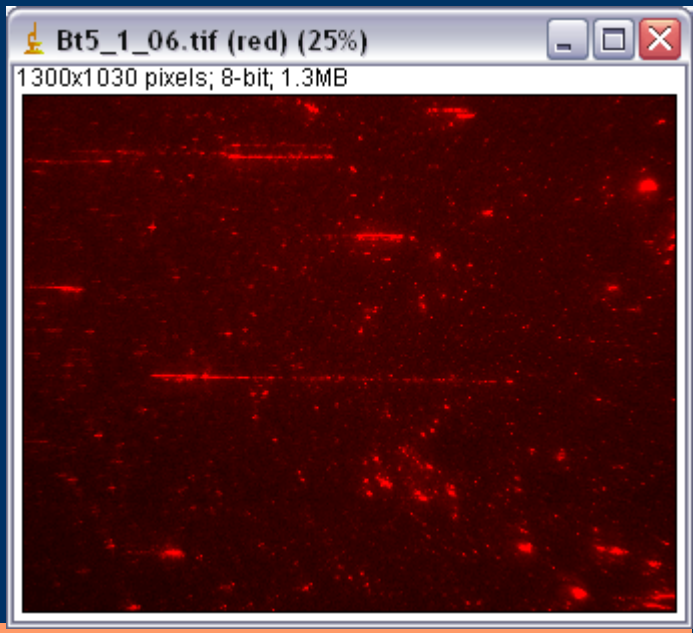
measure the lengths of the DNA molecules

measure the lengths of the replication sites within each DNA molecule

measure the distances between replication sites for each DNA molecule







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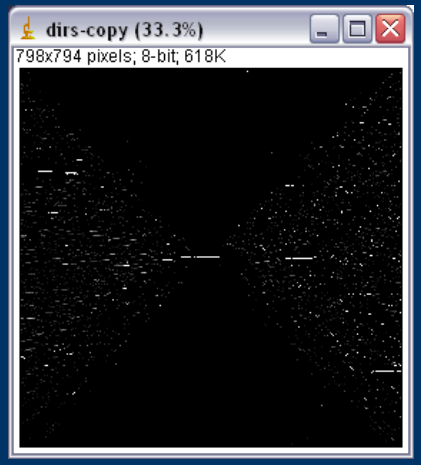
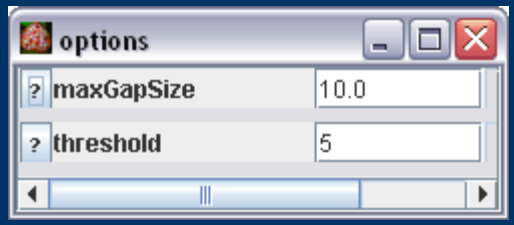
MRI Cell Image Analyzer – applications
2. dna combing – automatic solution



to find the green segments

- calculate hessian derivative
- threshold / find objects
- keep only “long” objects
- for remaining object centers
  - calculate shortest path to all pixels upto a distance
  - scan from the middle to the borders, allowing for gaps of max size g



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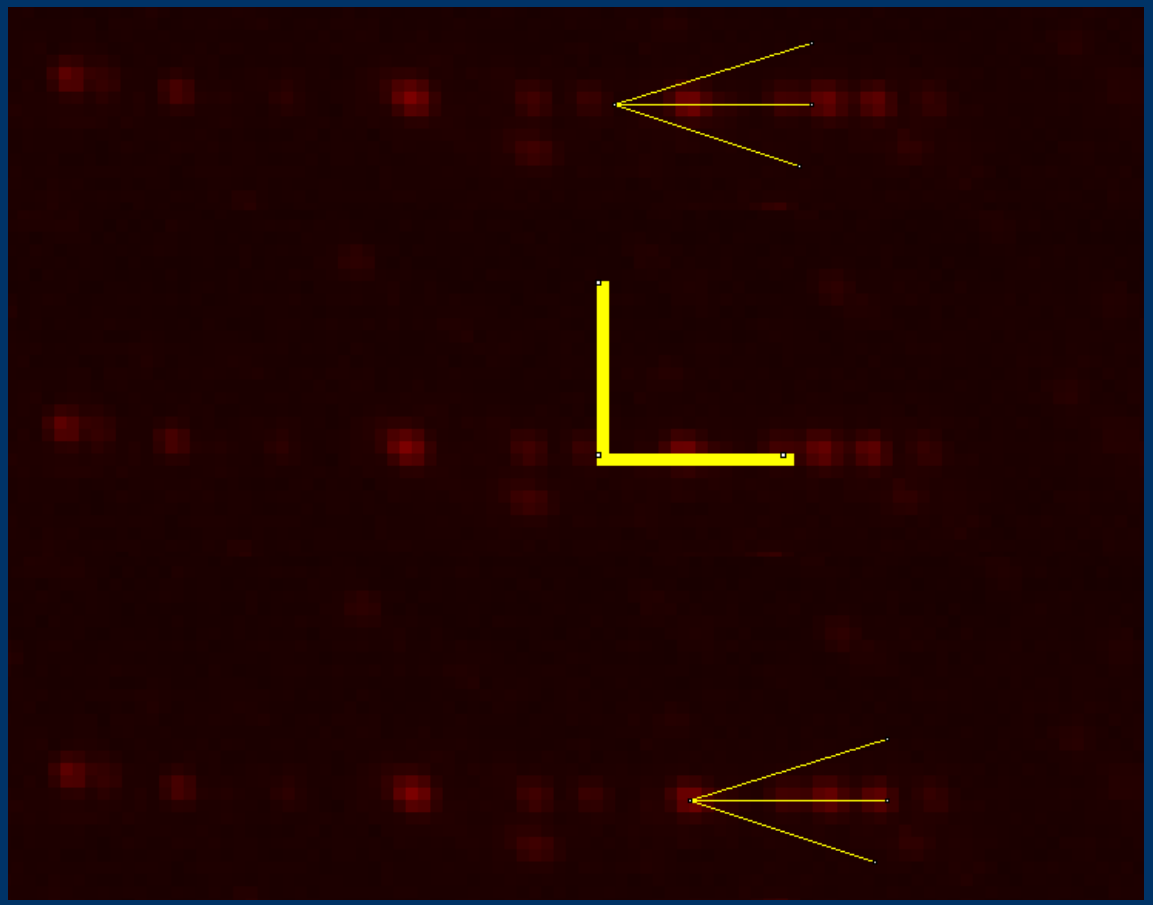
MRI Cell Image Analyzer – applications
2. dna combing – automatic solution



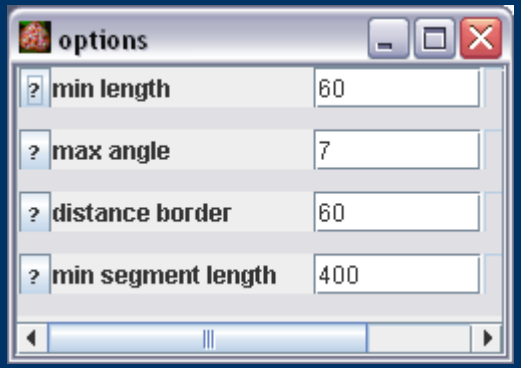
to find start and end of the molecule (red)

start in the middle of a green segment

find the best direction to go
(highest average intensity for a line segment of size s)

move one pixel in that direction, if intensity in a line segment in that direction is higher than in the perpendicular direction

else stop



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MRI Cell Image Analyzer – applications
2. dna combing – automatic solution

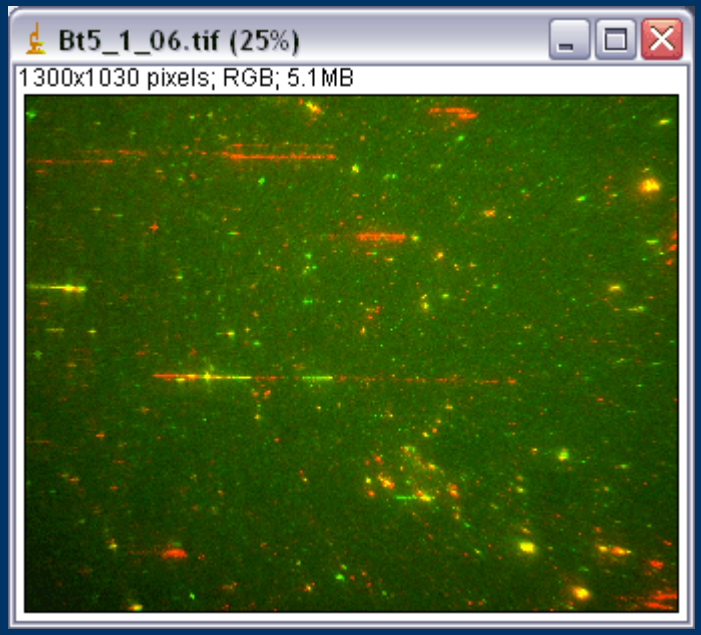
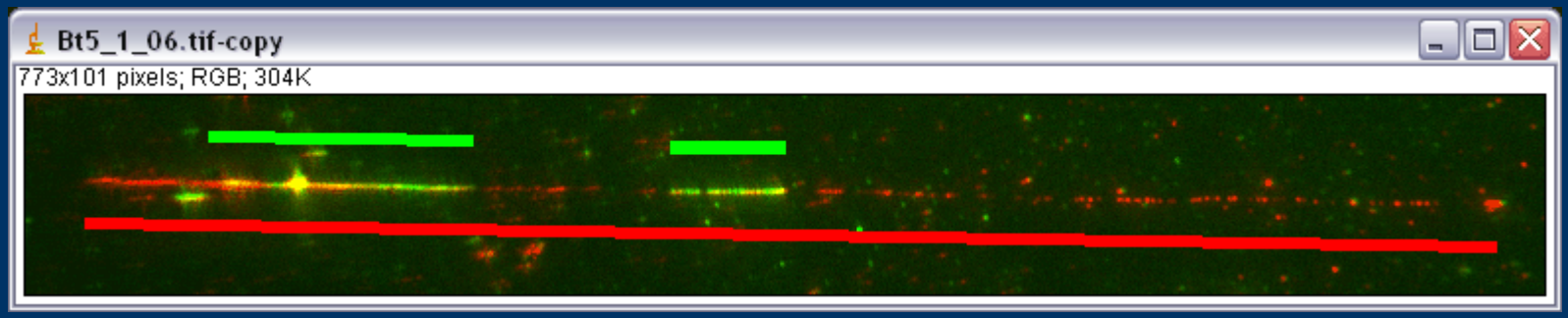


image	na brin n	start x	start y	end x	end y	total length	folder
Bt5_1_06.tif	1	254,86	559,48	973,72	570,55	718,95	Z:\baecker\combing\

1	2
318.0, 560.0, 453.0, 562.0	551.0, 564.0, 610.0, 564.0

red	green	red	green	red
63,14	135,01	98,02	59	363,78

1-2	2-3	3-4	4-5
195,02			



solution has still to be evaluated

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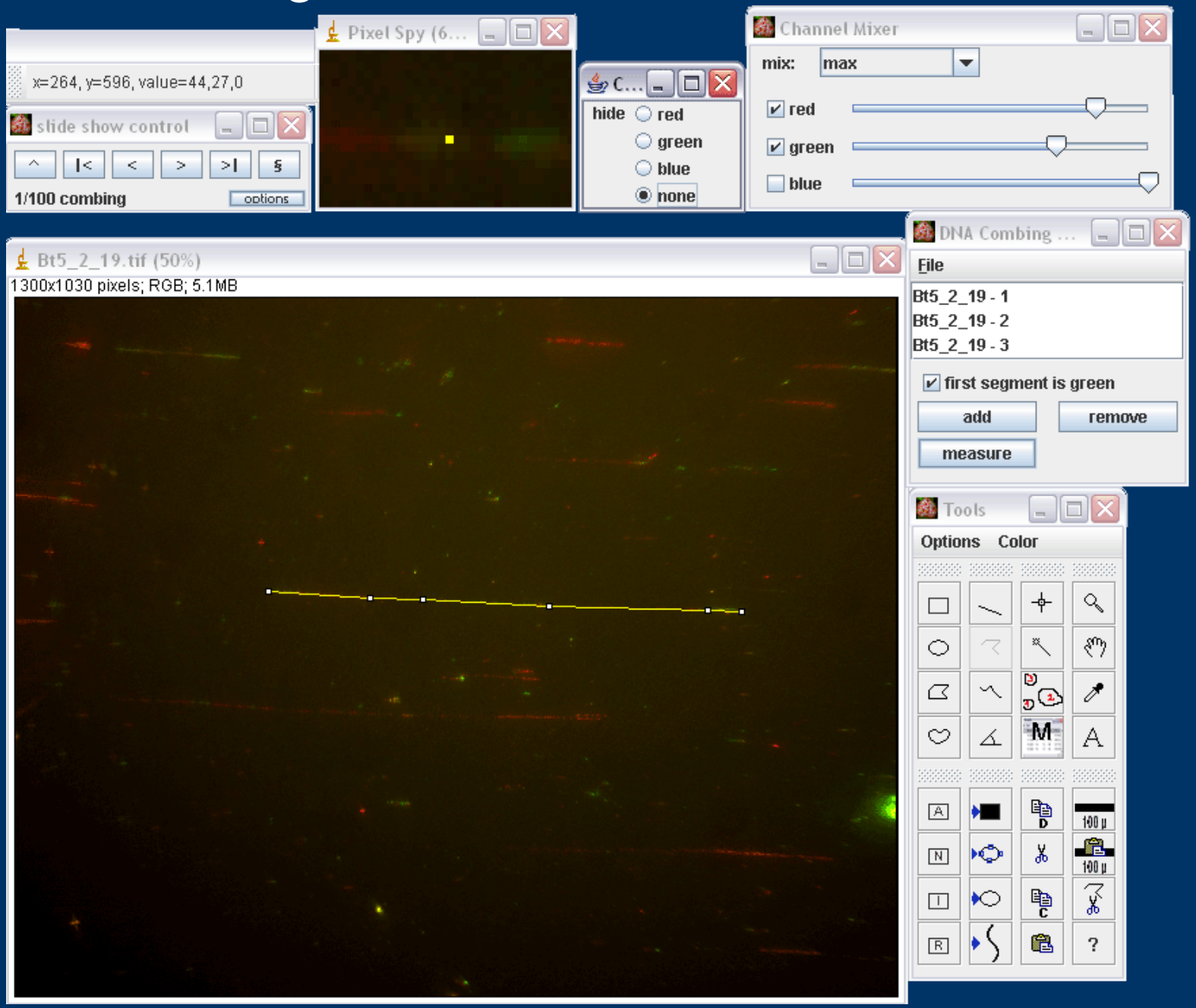
MRI Cell Image Analyzer – applications
2. dna combing – manual solution



use **slide show control** to select image

use **channel mixer** to adjust view

use **pixel spy** to see exactly where you set marks

use **polygon selection tool** to mark red and green segments

use **DNA combing tool** to save / load selections and to create reports

reports have the same format as in the automatic application

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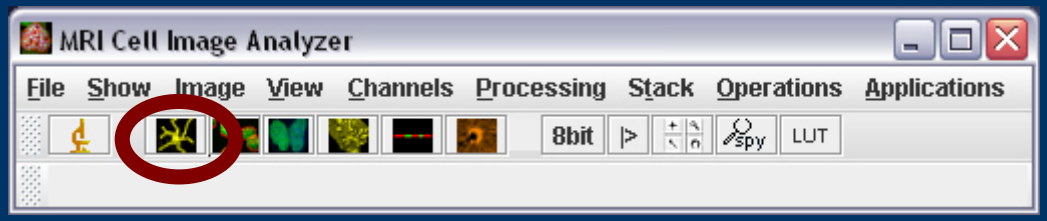
MRI Cell Image Analyzer – applications
3. neuronj



Conditions of usage
If you publish results that are based on NeuronJ, you are expected to acknowledge the work of Erik Meijering by putting a reference to the following paper:

* E. Meijering, M. Jacob, J.-C. F. Sarria, P. Steiner, H. Hirling, M. Unser, **Design and Validation of a Tool for Neurite Tracing and Analysis in Fluorescence Microscopy Images**, Cytometry, vol. 58A, no. 2, April 2004, pp. 167-176.

•MRI additions:
- slide show control to open next image with one click
  - automatically apply brighness / contrast adjustment
  - automatically apply lookup table
- you can directly work on 16bit images (automatic internal conversion)
- per default tracings are saved automatically when you change the image
- pixel spy to see the region under the pointer magnified
- rectangular selection tool, to auto adjust brighness / contrast based on region

delete all tracings
open image
save tracings

add tracing
delete tracing
measure
label tracings
parameters
zoom in / out
scroll
rectangular selection

exit
help

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MRI Cell Image Analyzer – applications
3. neuronj

open first image

slide show control opens automatically

adjust zoom

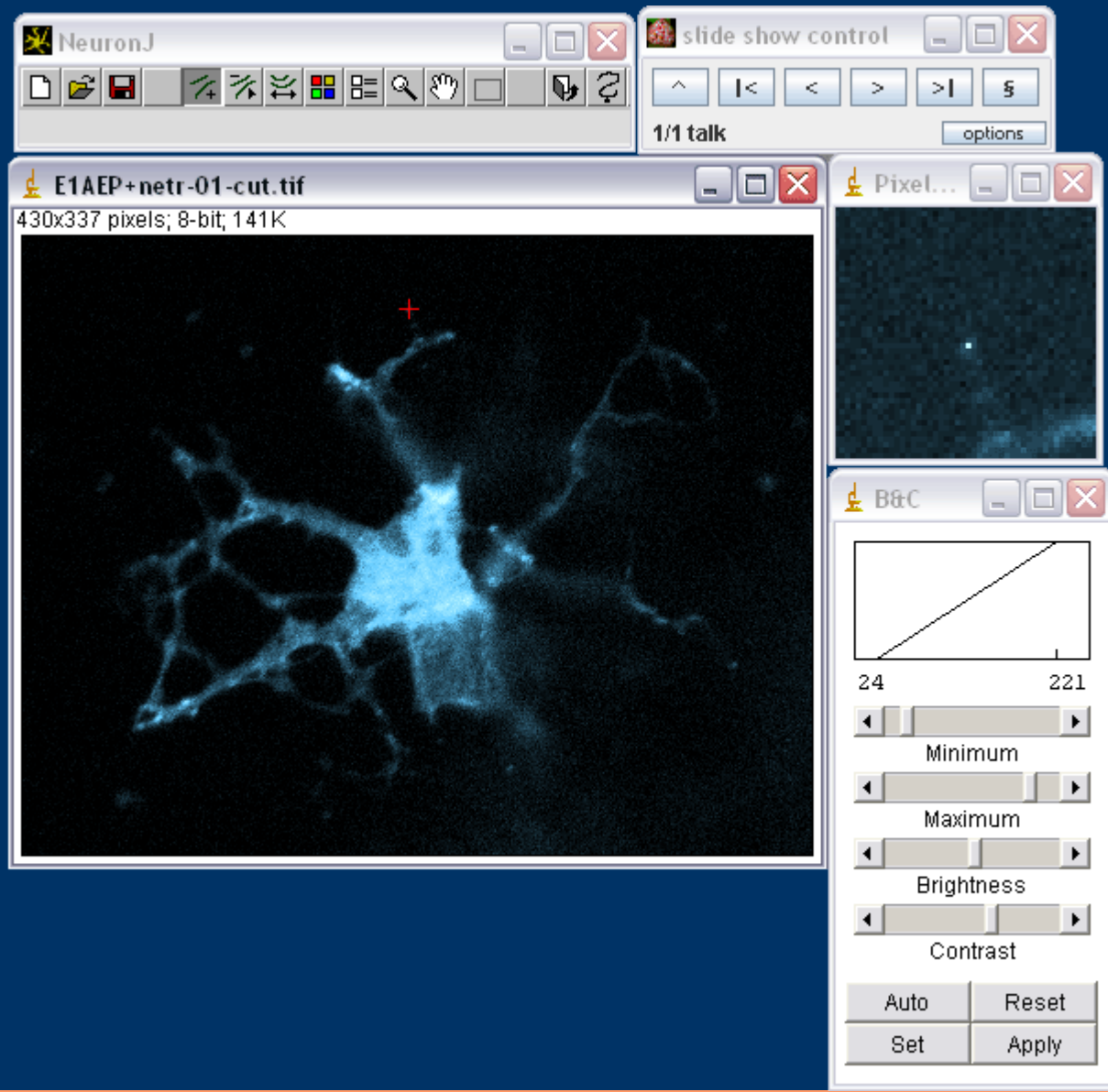
adust brightness contrast

click add tracing

go near the beginning of the neurite

the cursor snaps automatically to good starting points

left click to start first tracing



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MRI Cell Image Analyzer – applications
3. neuronj

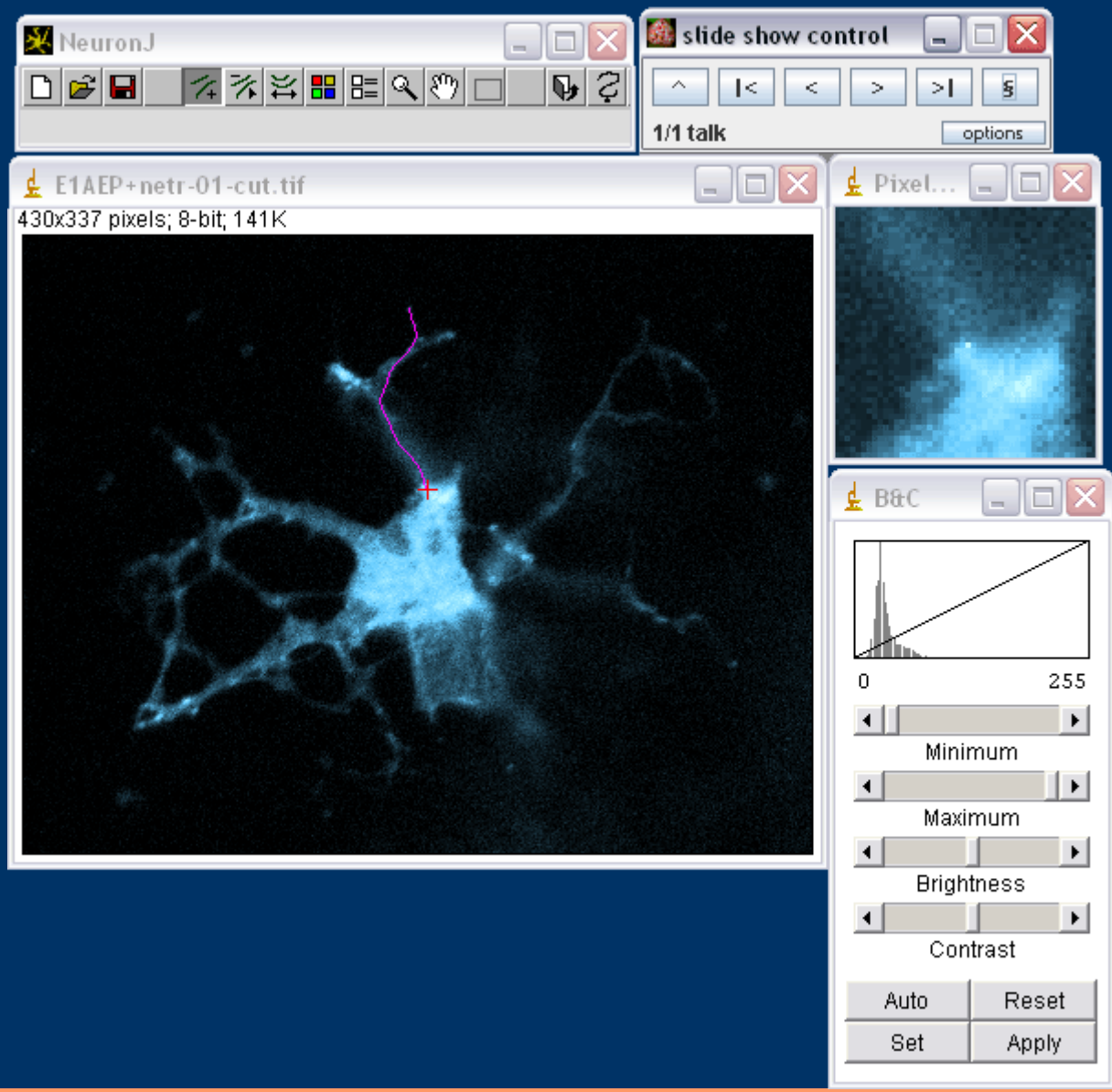
follow the neurite approximatively to the cell body

neuronj finds the neurite itself

if you see that the tracing takes a different way as expected at a crossing, left click to add intermediate point

press space bar to finish the tracing

trace all neurites you want to measure this way



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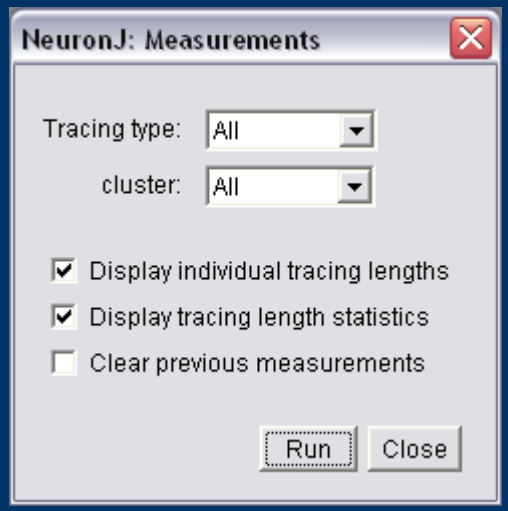
MRI Cell Image Analyzer – applications
3. neuronj

press measure to add the measurements of this image to the results table



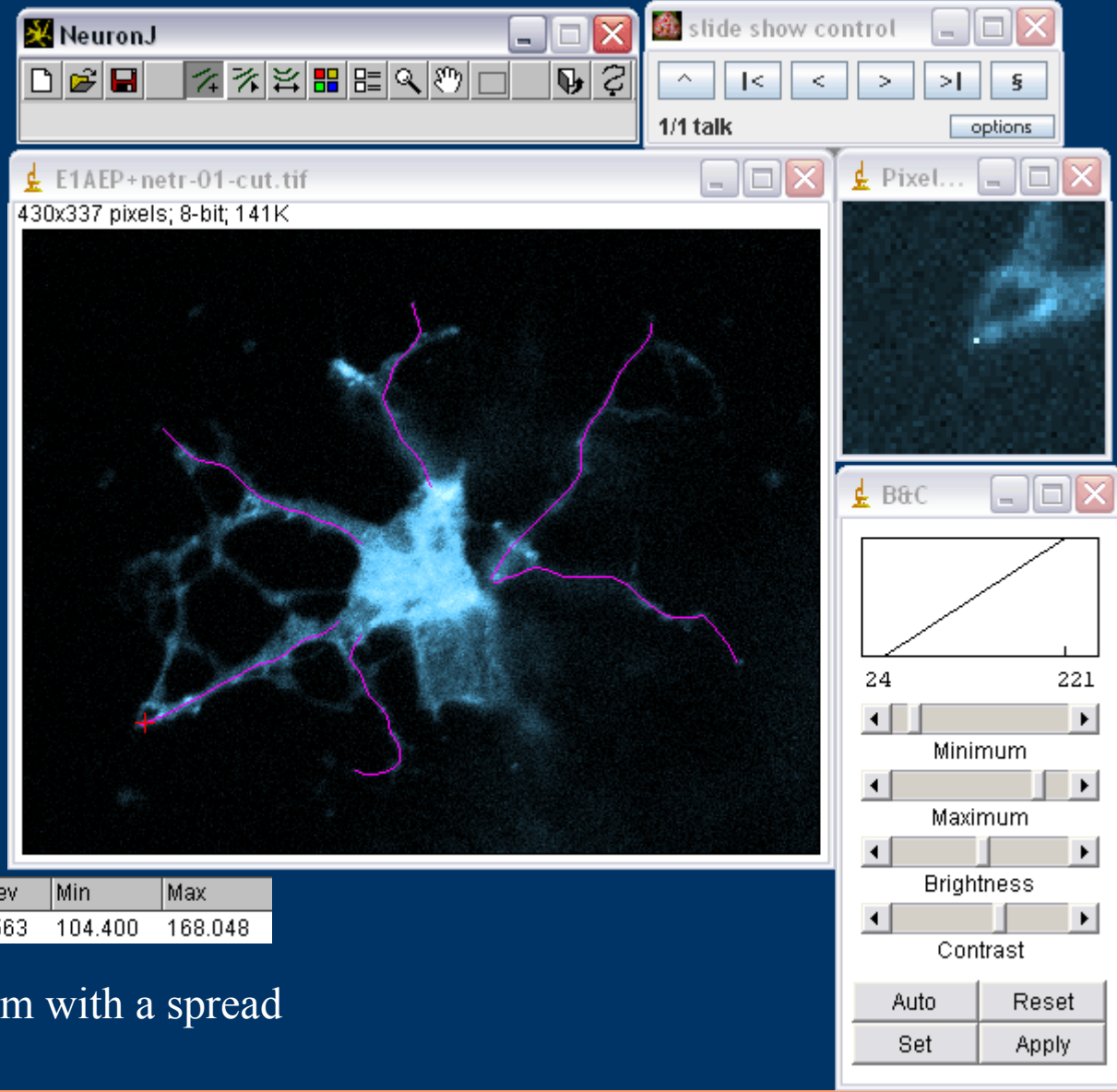


Image	Tracing	Cluster	Type	Label	Length
E1AEP+netr-01-cut	N1	Default	Default	Default	113.123
E1AEP+netr-01-cut	N2	Default	Default	Default	168.048
E1AEP+netr-01-cut	N3	Default	Default	Default	121.976
E1AEP+netr-01-cut	N4	Default	Default	Default	127.052
E1AEP+netr-01-cut	N6	Default	Default	Default	153.822
E1AEP+netr-01-cut	N7	Default	Default	Default	104.400

Image	Cluster	Type	Count	Sum	Mean	StDev	Min	Max
E1AEP+netr-01-cut	All	All	6	788.421	131.403	24.563	104.400	168.048

you can save the result tables and open them with a spread sheet program, or use copy and paste

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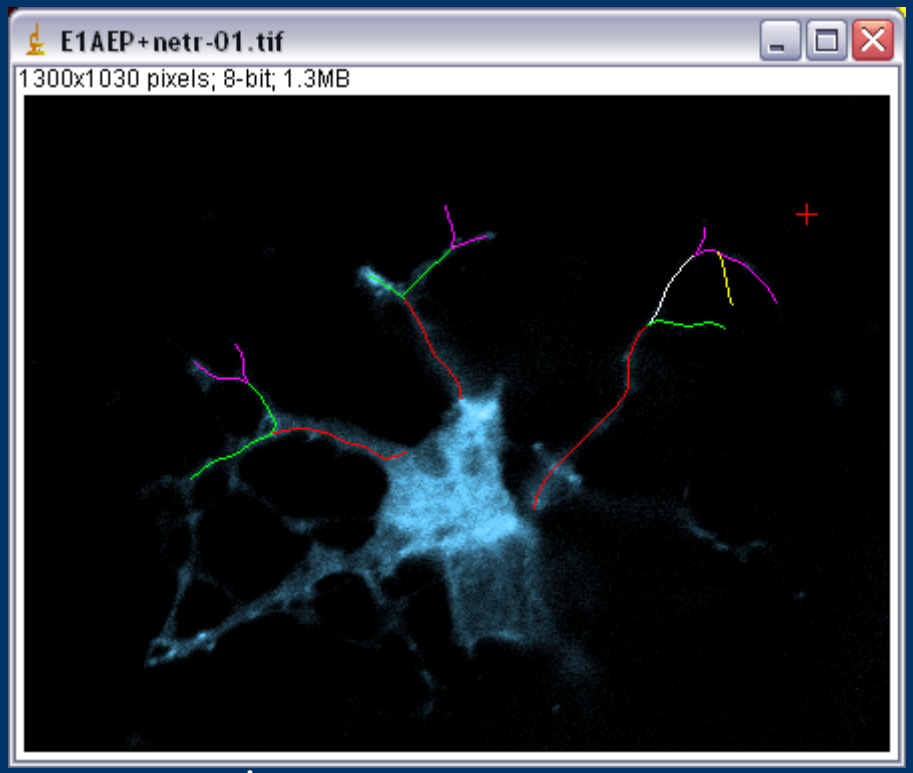
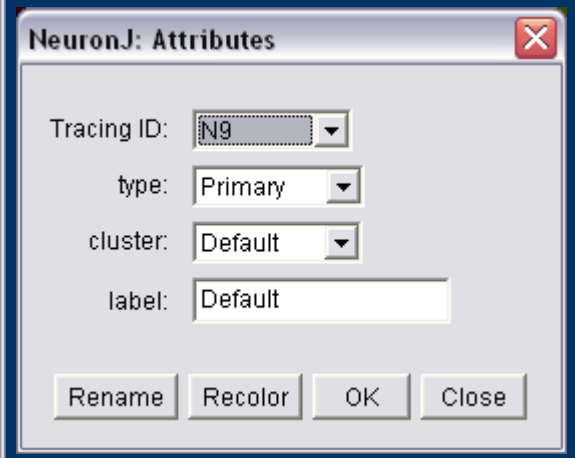
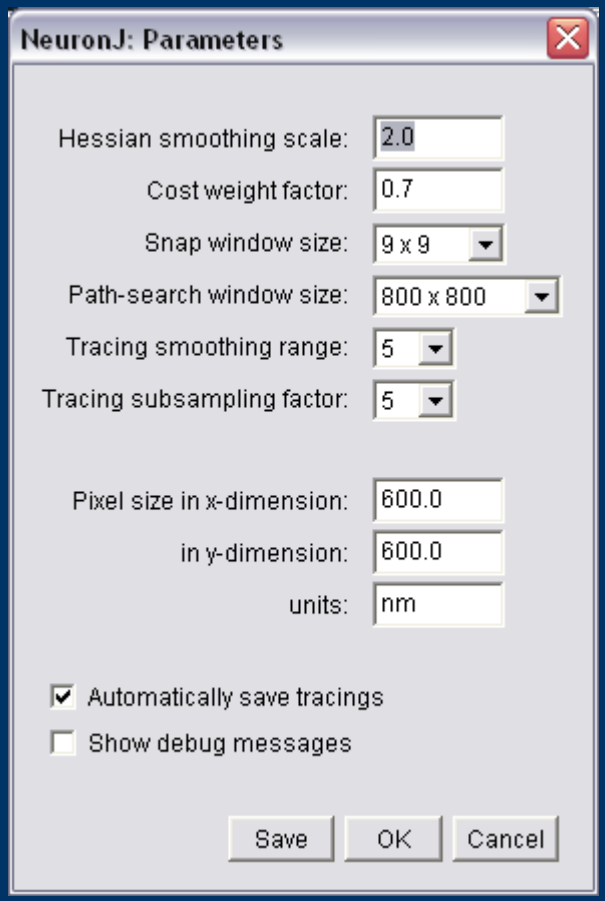
MRI Cell Image Analyzer – applications
3. neuronj







- group tracings
  - different types of neurites
    - axon, dendrite, primary, secondary, ...
    - or create your own types
  - different clusters
  - label tracings
- calibrate the spacial dimensions
  - measure in nanometer, micron, etc.

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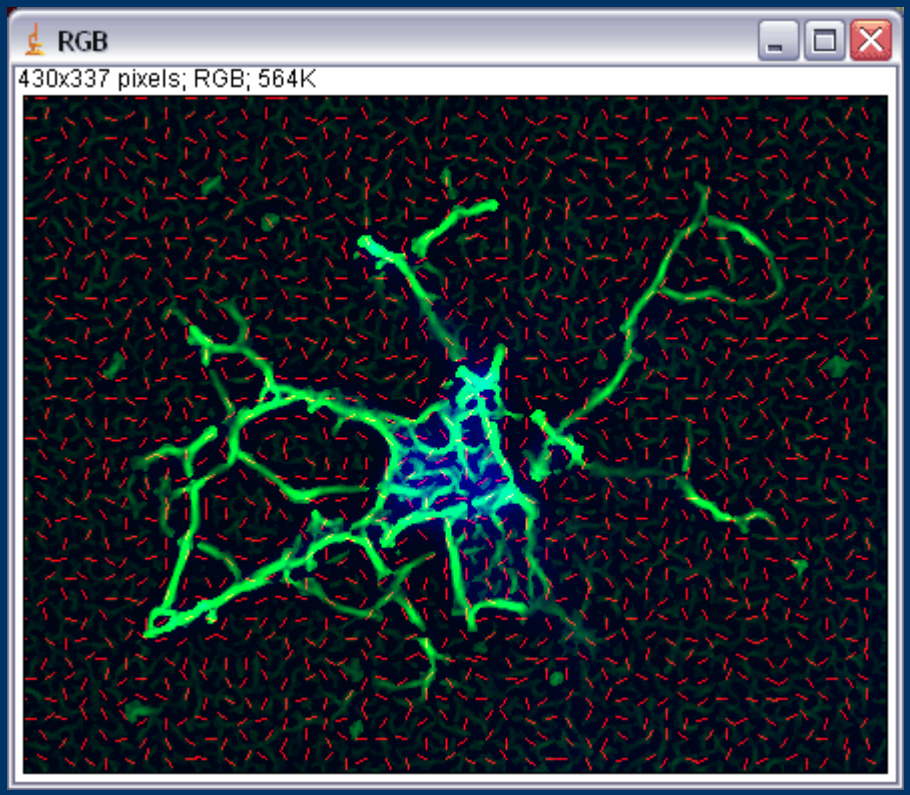
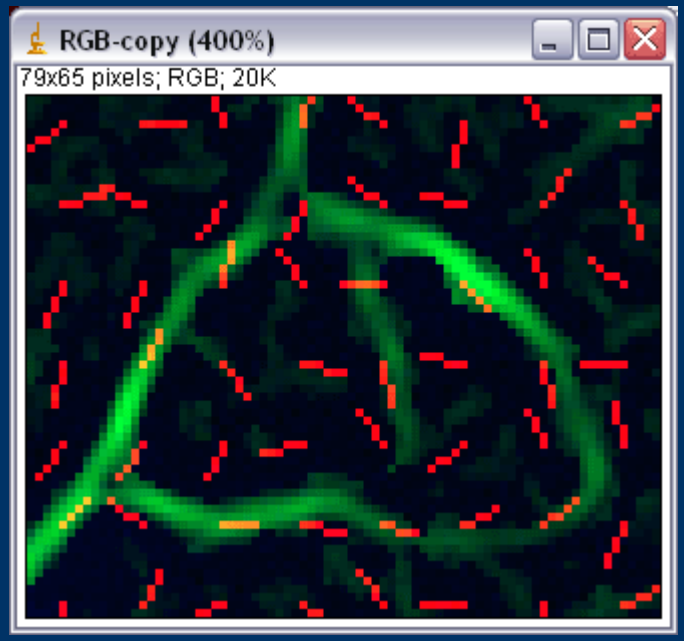
MRI Cell Image Analyzer – applications
3. neuronj

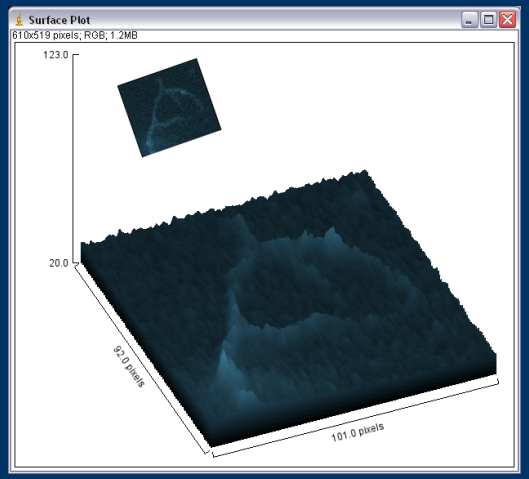




Imagine
- image as landscape
- light regions as mountains

neurites become ridges

compute cheapest path
- from start point
- to mouse pointer



use second order differential operator to get
- directions of ridges
- a likeliness value for each pixel to belong to a neurite
- by comparing the magnitutes of the eigenvalues

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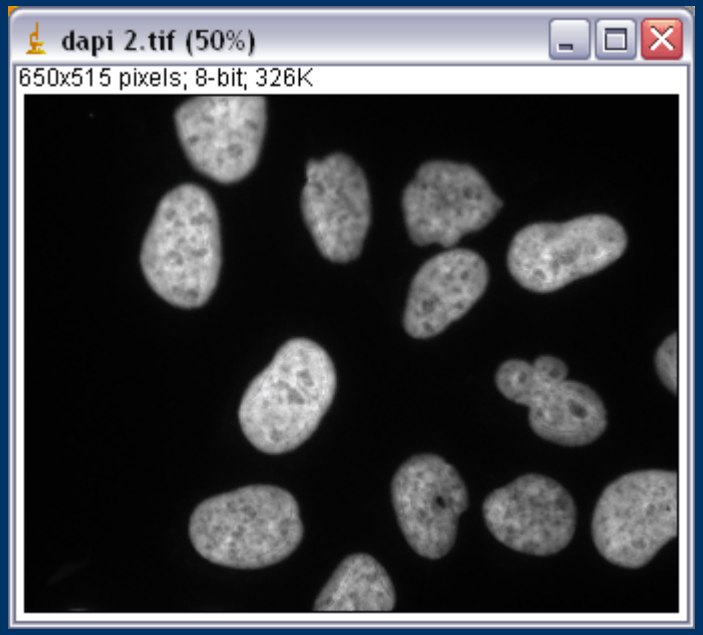
MRI Cell Image Analyzer – applications
4. comparing intensities



what is the proportion of fluorescence between nuclei and cytoplasm in the first image?

the second image is used to identify the nuclei

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MRI Cell Image Analyzer – applications
4. comparing intensities

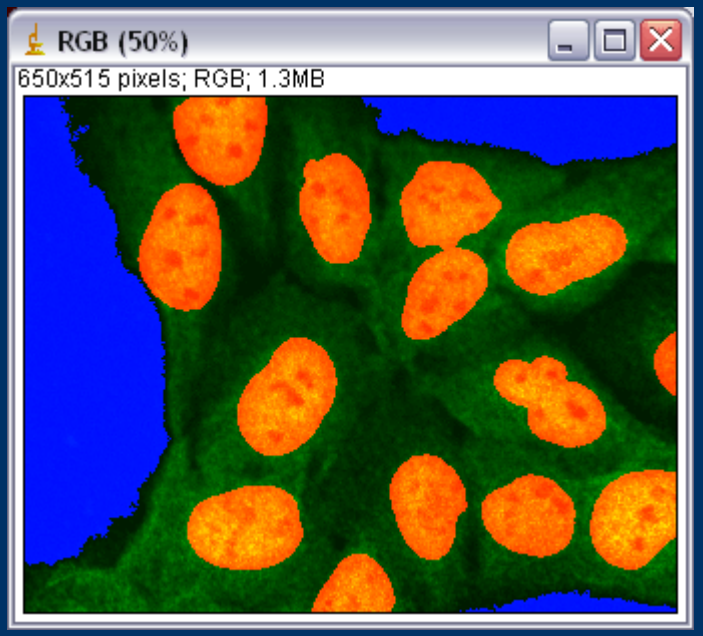
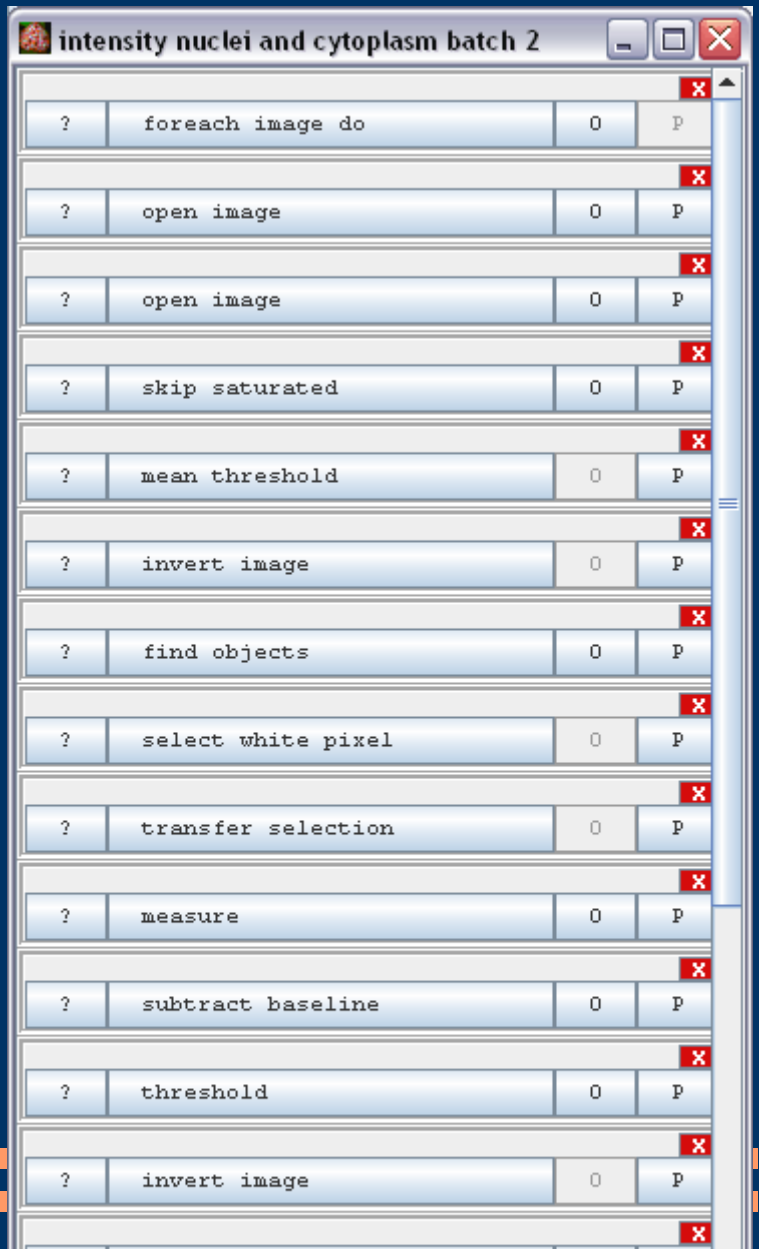


image	icn factor	percent nuclei	percent cytoplasm	av. nuclei intensity	av. cytoplasm intensity
dapi 2.tif	0,85	0,46	0,54	112,67	61,99

- threshold nuclei image
- select objects
- transfer selection to input image
- measure selection
- subtract adaptive baseline to zero background
- select none zero pixels of the image in the original image
- measure
- subtract nuclei intensity
- calculate proportions



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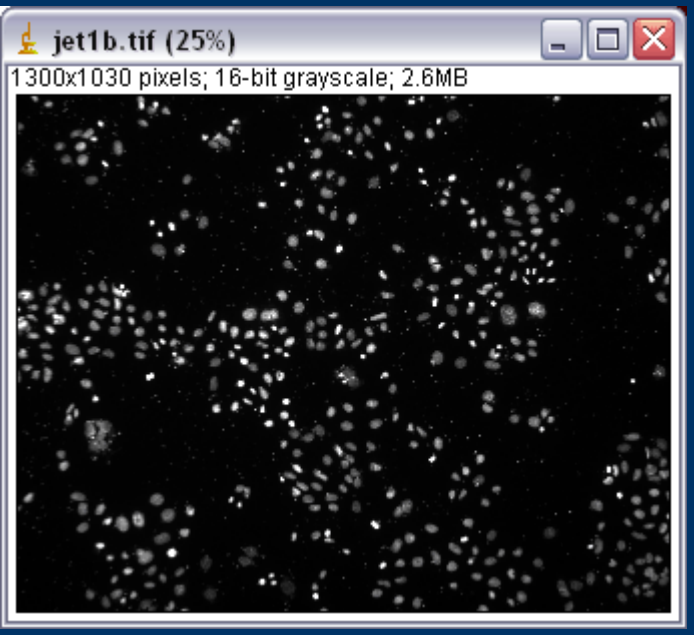
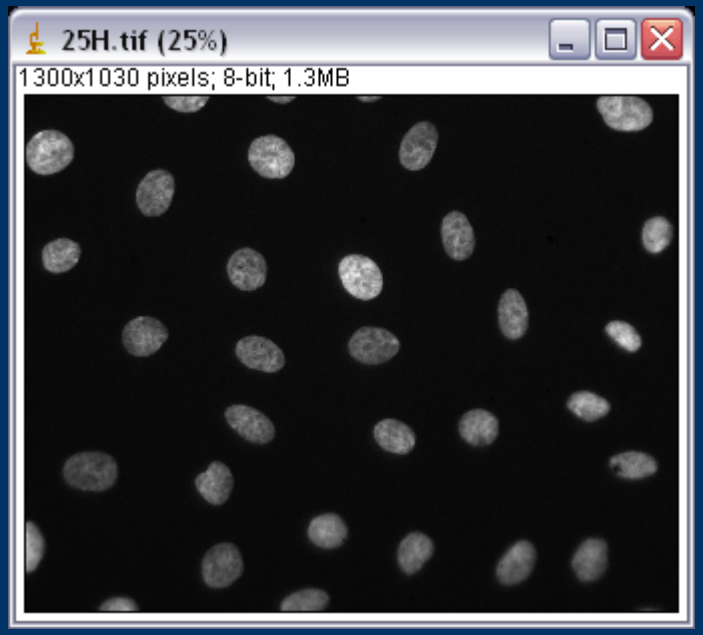
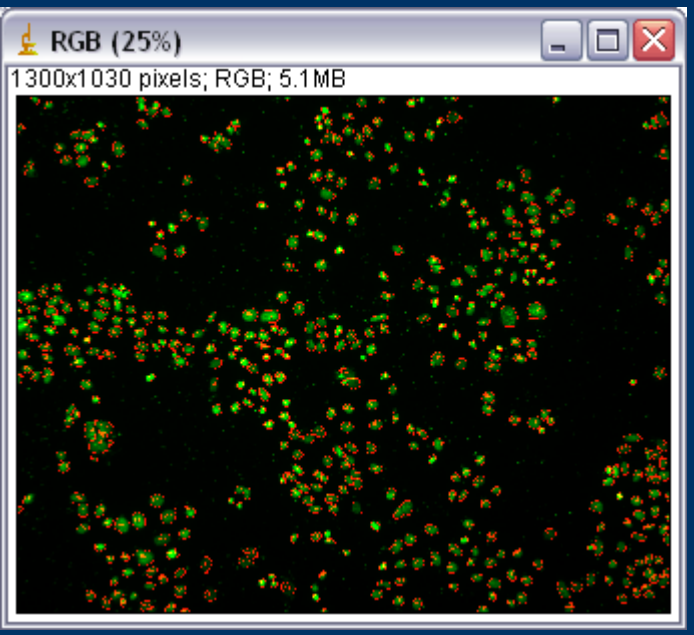
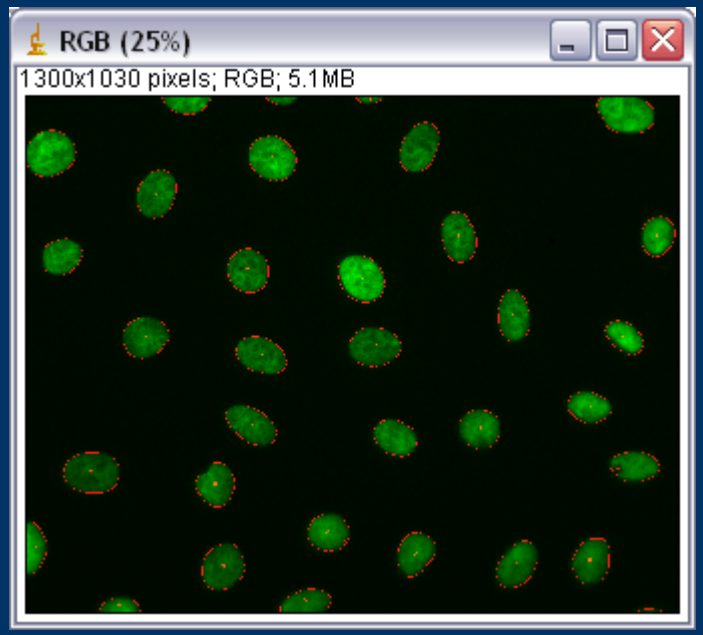
MRI Cell Image Analyzer – applications
5. counting cells or nuclei









How many cells are there?

image	number of objects	folder	
25H.tif	34	E:\besnard\	
jet1b.tif	495	E:\etienne	delepine\

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MRI Cell Image Analyzer – applications
further applications

further applications

- count and measure marked regions in nuclei
- create compressed quick time movies from time series of arbitrary size
- create film with overlay of phase contrast and fluorescence image

work in progress

- particle tracking – measure velocity of moving cells
- measure size of changing objects in time series
- counting different cell types (normal, apoptotic, etc.)

summary and outlook

MRI Cell Image Analyzer – conclusion and outlook

- MRI Cell Image Analyzer
  - is an adequate tool for the rapid devolpment of image analysis applications
  - finding a solution and creating the application go hand in hand with it
  - it can be used by biologists and application developpers together
  - batch applications can treat all images to be analyzed in one run
  - since it is based on imagej a lot of functionallity is immediately available, including plugins for specific tasks.
- Sometimes automatic solutions are not (yet) adequate
  - the possibility for the user to make corrections has to be build in
  - or at least appropriate tools for the manual treatment can be provided
- A number of image analysis applications has been realized.

  Showing the value of the bottom up approach
    - where specific problems are solved and
    - solutions are assembeled to a framework

So let us try to solve your image analysis problem now...

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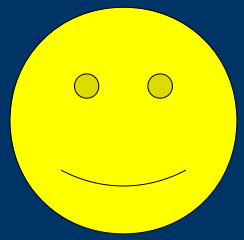
MRI Cell Image Analyzer – conclusion and outlook

- what comes next depends on you...
- there seems to be a need for a virtual stack processing
  - to treat very big sequences (stack, time series)
  - virtually like any other images without the need to load them entirely
  - only showing data necessary at one moment
  - working on the disk behind the scenes
  - the concept is known from text, sound and movie editing and is called a streaming editor there

MRI – announcement

- for doing deconvolution at MRI
  - you will soon be able to upload your images to our fileserver and to download results
  - this will be possible from all MRI analysis pcs
  - if you want to do it from other pcs, for example in your lab
    - please tell us
      - we need to know the ip address
      - we need to install and configure a client software

Thank you for your attention!



? Questions ?

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MRI Cell Image Analyzer – literature and links

ImageJ

Abramoff, M.D., Magelhaes, P.J., Ram, S.J. "Image Processing with ImageJ". Biophotonics International, volume 11, issue 7, pp. 36-42, 2004.

Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://rsb.info.nih.gov/ij/ , 1997-2005.

Online Manual for the WCIF-ImageJ collection, ImageJ for microscopy, Image Processing and Analysis in Java, http://www.uhnresearch.ca/facilities/wcif/imagej/

Visual programming

Visual Programming Research, Introduction and Philosophy, Dr. R. Mark Meyer, Computer Science Department, Canisius College, July 1, 1999, http://www-cs.canisius.edu/~meyer/VP/intro.html

Image Processing

Look Up Tables - HyperMedia Image Processing Reference, The University of Edingburgh, Look Up Tables and Colourmaps, http://www.cee.hw.ac.uk/hipr/html/colmap.html

Histogram - HyperMedia Image Processing Reference, The University of Edingburgh, Intensity Histogram, http://www.cee.hw.ac.uk/hipr/html/histgram.html

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MRI Cell Image Analyzer – literature and links

Image Processing

Contrast Stretching - HyperMedia Image Processing Reference, The University of Edingburgh, Contrast Stretching,, http://www.cee.hw.ac.uk/hipr/html/stretch.html

Thresholding - HyperMedia Image Processing Reference, The University of Edingburgh, Thresholding,, http://www.cee.hw.ac.uk/hipr/html/threshld.html

Image Arithmetic - HyperMedia Image Processing Reference, The University of Edingburgh, Image Arithmetic,, http://www.cee.hw.ac.uk/hipr/html/arthops.html

color models (RGB, CMYK) – Wikipedia, Color space, http://en.wikipedia.org/wiki/Color_space

Gaussian Blur – Algorithms, Ian Craw and John Pulham, University of Aberdeen, http://www.maths.abdn.ac.uk/~igc/tch/mx4002/notes/node99.html

Dilation - HyperMedia Image Processing Reference, The University of Edingburgh, Dilation,, http://www.cee.hw.ac.uk/hipr/html/dilate.html

Autothreshold – wikipedia, Thresholding (image processing), http://en.wikipedia.org/wiki/Thresholding_(image_processing)

MRI Cell Image Analyzer – literature and links

Hessian - IEEE TRANSACTIONS ON IMAGE PROCESSING, VOL. 7, NO. 3, MARCH 1998 353, Adaptive Smoothing Respecting Feature Directions, Ren´e A. Carmona, *Member, IEEE,* and Sifen Zhong (http://www.mi.uib.no/~mariusl/carmona.pdf)

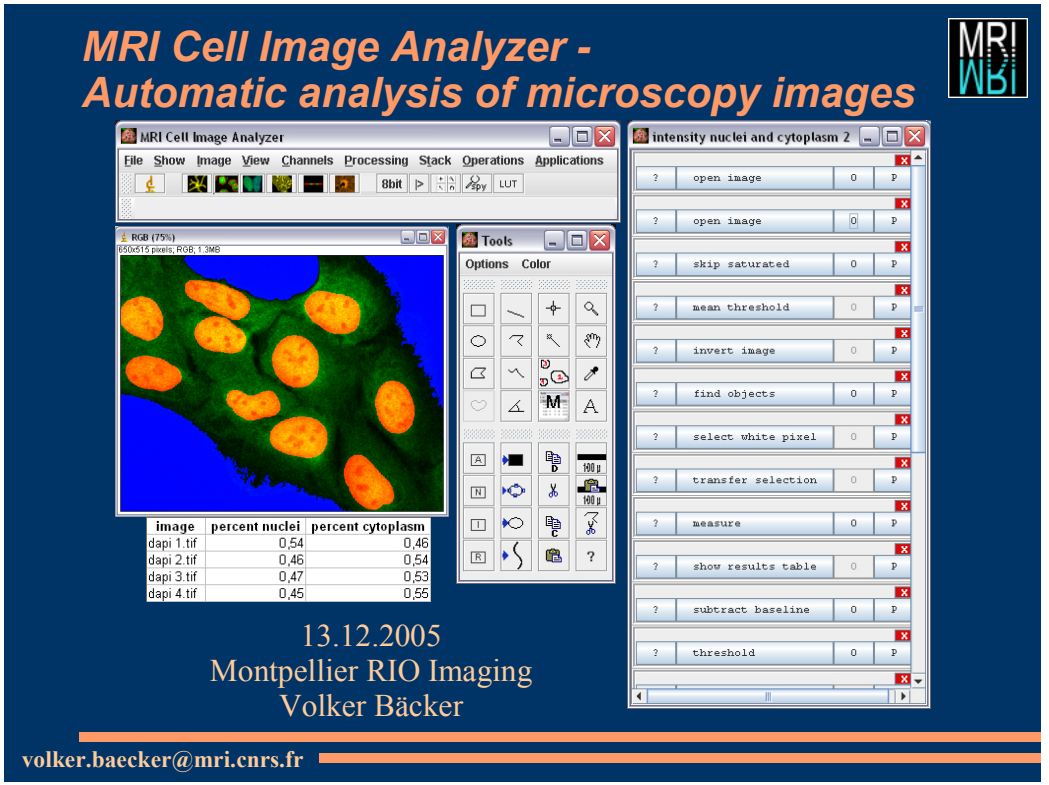
Pattern Recognition

H. Niemann: Klassifikation von Mustern. Springer Verlag, Berlin, 1983. (http://www5.informatik.uni-erlangen.de/Personen/niemann/klassifikation-von-mustern/m00links.html)

R.O. Duda, P.E. Hart: Pattern Classification and Scene Analysis. John Wiley & Sons, Inc., 1973.

NeuronJ

E. Meijering, M. Jacob, J.-C. F. Sarria, P. Steiner, H. Hirling, M. Unser, Design and Validation of a Tool for Neurite Tracing and Analysis in Fluorescence Microscopy Images, Cytometry, vol. 58A, no. 2, April 2004, pp. 167-176. (http://imagescience.bigr.nl/meijering/publications/abstracts/cyto2004.html)

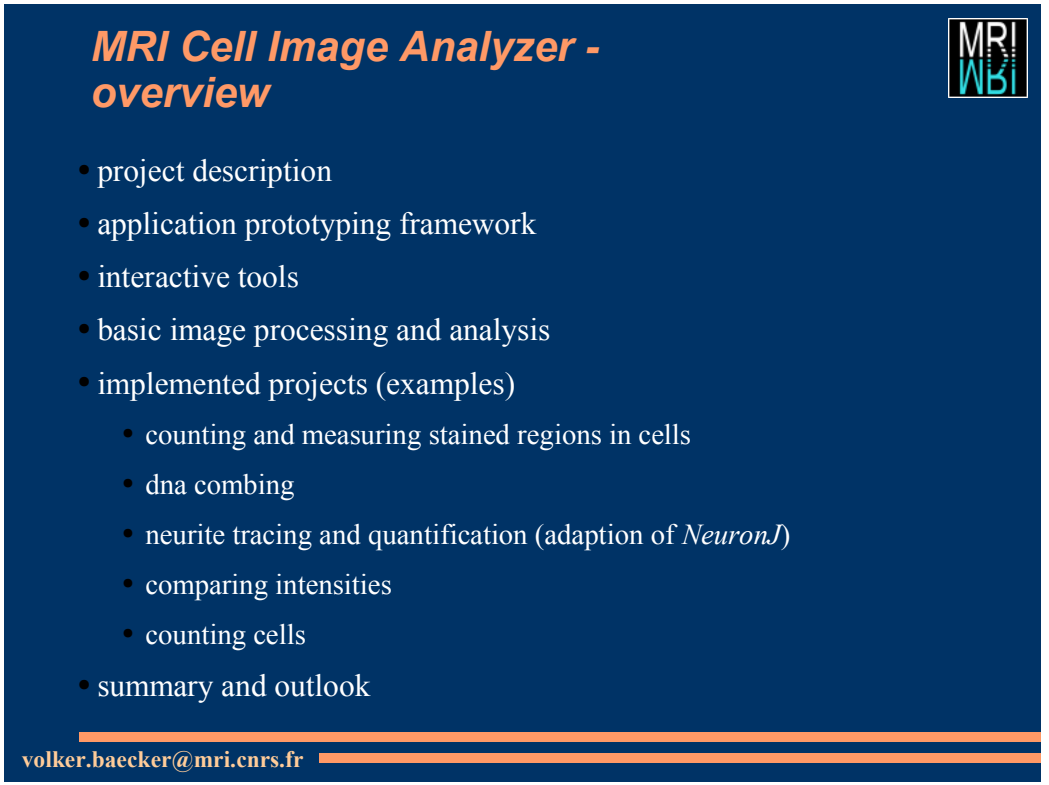


Welcome to my talk about MRI Cell Image Analyzer.

My main project in the last six month was to create solutions for image processing and analysis tasks in the field of fluorescence microscopy imaging. And especially the automation of tasks in this area. This will be an ongoing activity in the future.

The approach was the following:
Starting with a concrete need of a research group a solution is developped.
The operations created for each solution are embedded into an image analysis and processing framework.

I'm going to present to you some of the projects realized and the framework developped along today.



I begin by explaining the requirements of the projects. Why we saw a necessity for it and how it differs from other solutions.

Then I present the framework for developping automatic image analysis applications that has been developped.

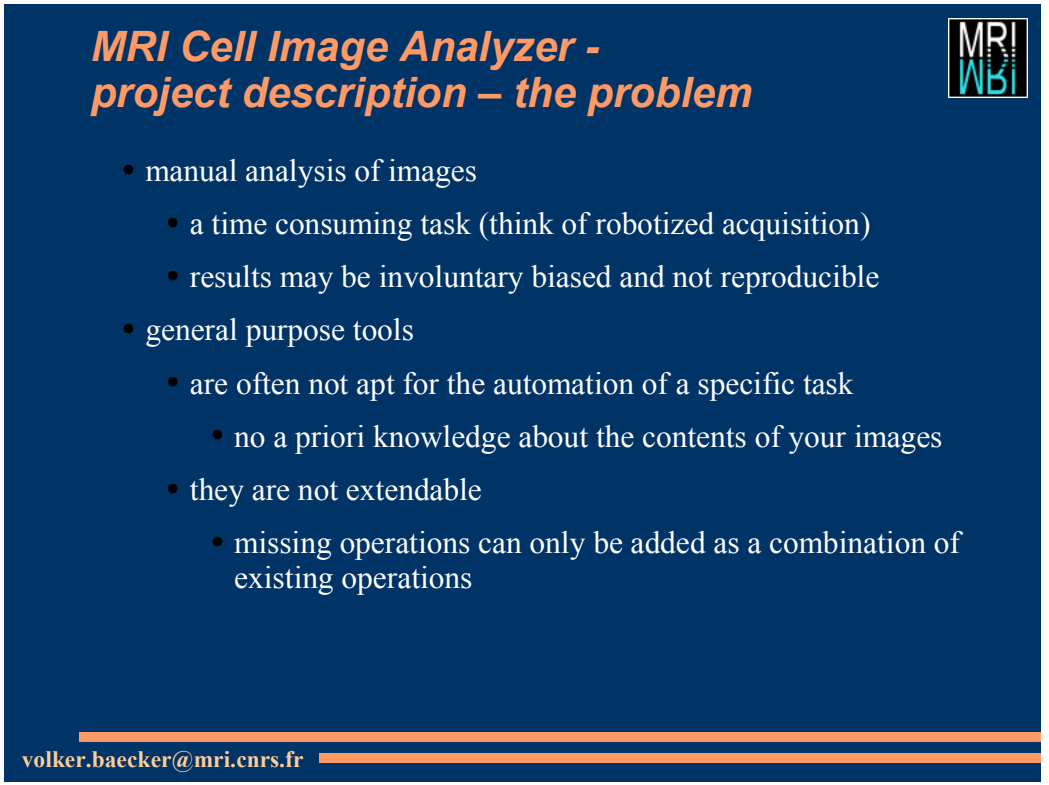
Although the focus is on the automation of tasks, interactive tools are needed in the process of finding a solution for a given problem. I'll give a short overview of the tools available in the envirnment and the tools I added.

I give a short introduction of what image processing and analysis means. And show with the help of one example how solutions can be developped.

I'll present the applications that have been realized.

In the end I summerize the work that has been done and give an outlook in which directions the work might continue in the future.

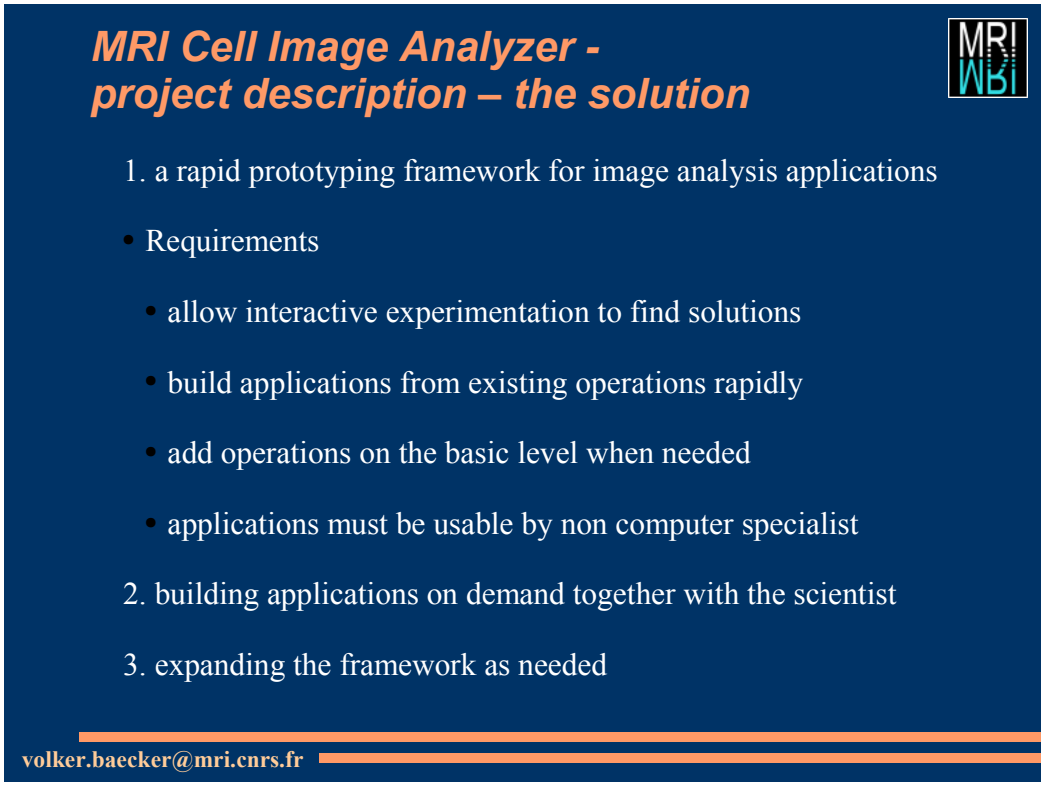
We start with a description of the project.



First:
Manual analysis of images can be a laborous and time consuming task.
With automatic acquisition you can create very big data sets without too much effort. This is fine because it allows you to do new kinds of experiments and observations. However to be useful the acquired data has to be analysed afterwards to get the answer to the question under examination. Thus automatic analysis and the rationalization of manual analysis are needed.

Second:
The available tools are doing a great job for the tasks they were made for. However they are general purpose, providing solutions for most common tasks. They can´t take into account knowledge about your special experiment that might be essential for doing the required analysis.

If you need some functionallity not provided you can´t simply ask your informatique expert of choice to add it. You have to ask the producer and wait for a new release of the software.



What is needed is a rapid prototyping framework that allows interactive experimentation to find solutions to image analysis problems.

That allows to build applications from existing operations rapidly.

That allows to add new operation on the programming language level when needed.

That provides a reasonably simple user interface for the end user to set parameters and start applications.

When you need to do image analysis, we will first check if it can be done with the available standard tools.

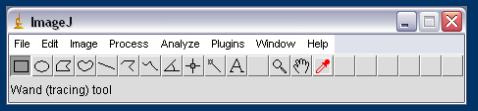
If not we´ll search a specific solution for the specific problem and implement it within the framework, extending the framework when necessary.

With each project the tools and operations are added to the framework that might be usefull for other projects.

MRI Cell Image Analyzer - project description – design decision

don't reinvent the wheel !

- base MRI-CIA on which image analysis library / kit ?
- ImageJ, because (Wayne Rasband, National Institute of Mental Health, Bethesada, Maryland, USA)
  - it has been created for the treatment of microscopy images
  - provides a solid image processing/analysis framework
  - an abundance of plugins for specific tasks available
  - a vivid user community
  - good documentation
  - it is public domain



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It was clear that I wouldn´t want to start at zero, reimplementing basic functionallity like reading of image file formats, showing images on the screen, etc. that already exists.

So I searched for a library / toolkit that provides the basic image processing functionallity.

I found that ImageJ was the right basis for the project.

Unlike other frameworks it has been created for the treatment of microscopy images

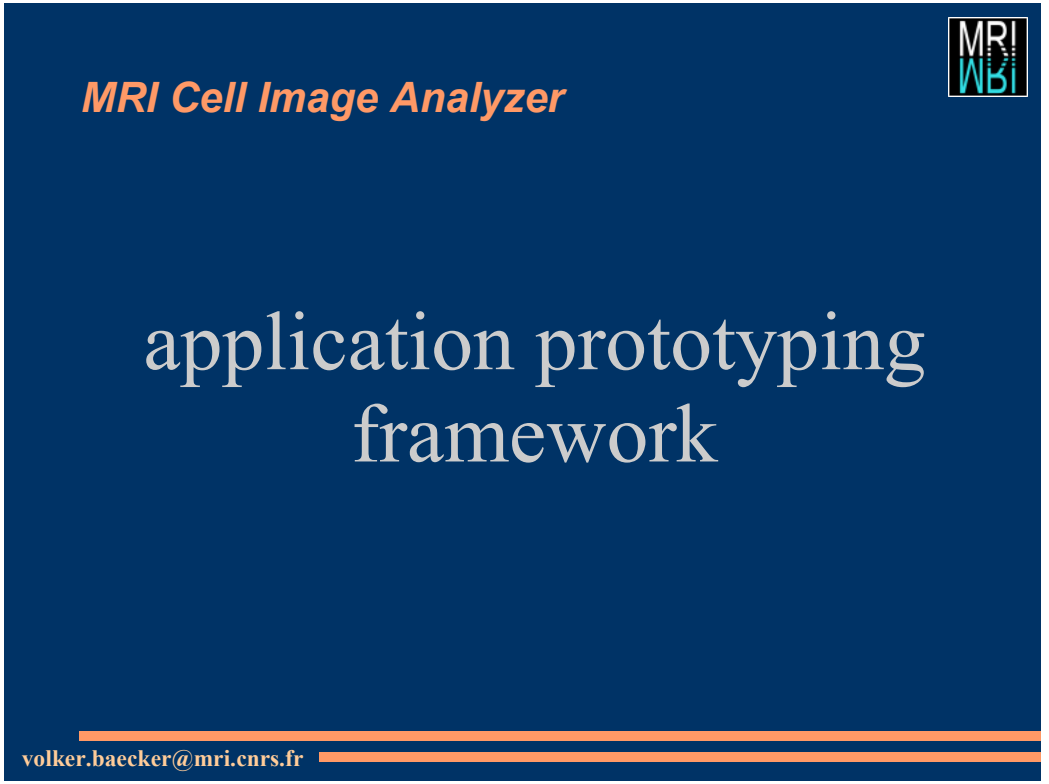
It provides a solid image processing and analysis framework.

A lot of plugins for specific tasks are available.

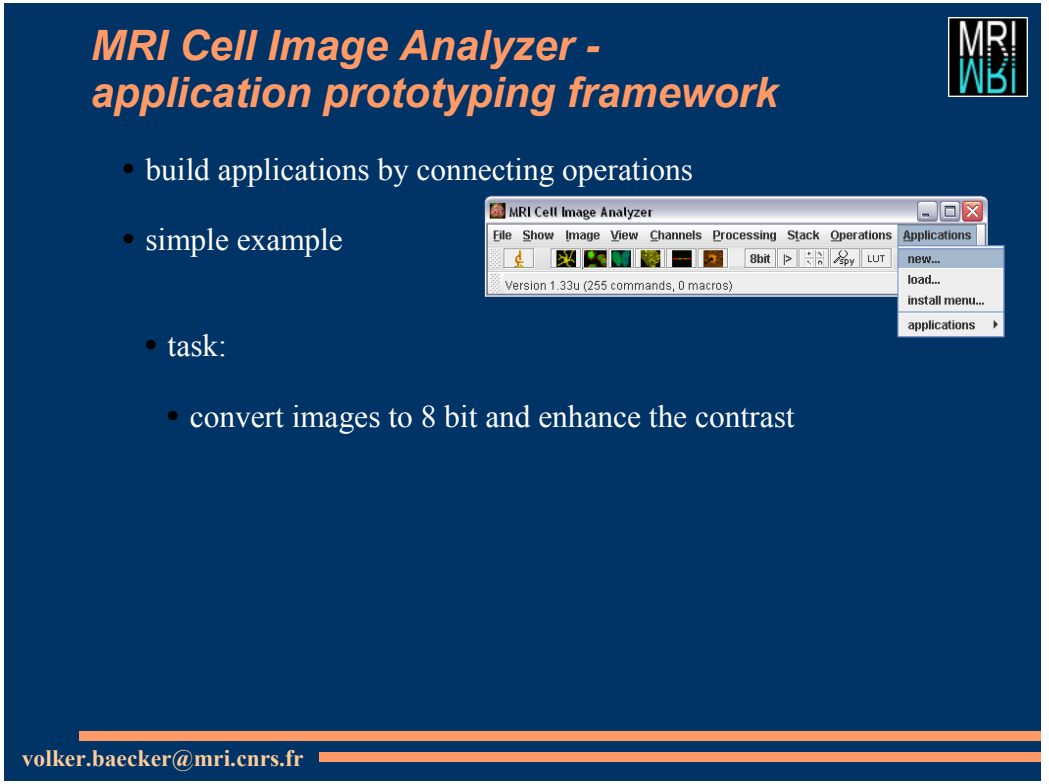
It is alive today.

Good documentation is available.

It is public domain and the source code is available, allowing to make changes when necessary.



Let's see how applications are created from existing operations, using MRI Cell Analyzer



Let´s have a look at a simple example.

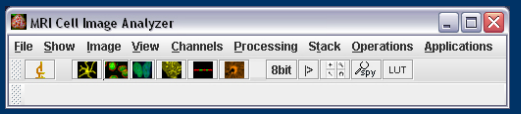
Imagine we want to convert a number of images to 8 bit and enhance the contrast in the same time so that the image is directly visible, without modifying the way it is displayed

To create a newv application

go to the menu application and select the entry new

MRI Cell Image Analyzer - application prototyping framework

- build applications by connecting operations
- simple example



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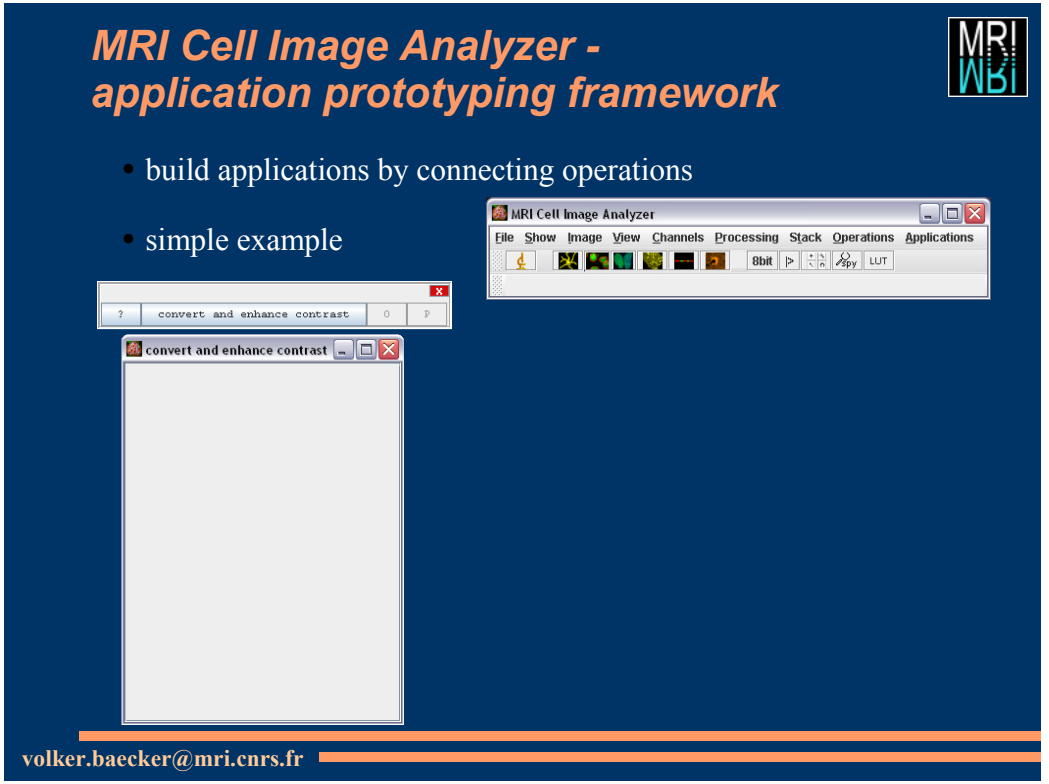
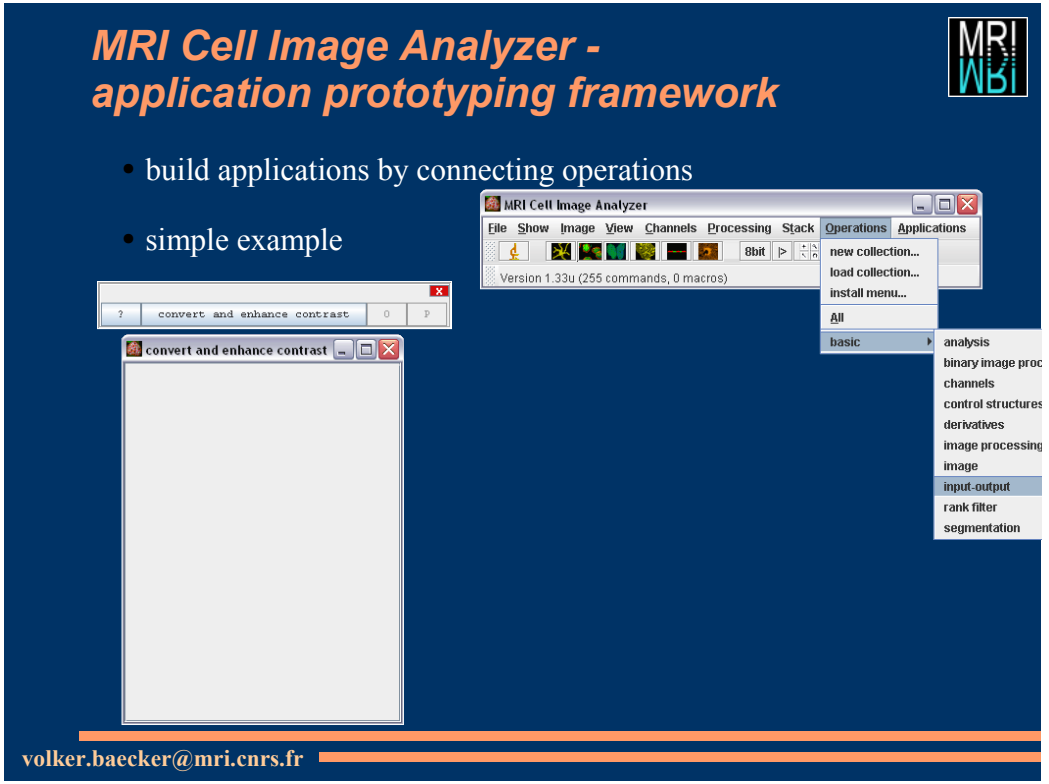
Enter the name of the new application in the dialog. Let´s call it “convert and enhance contrast”

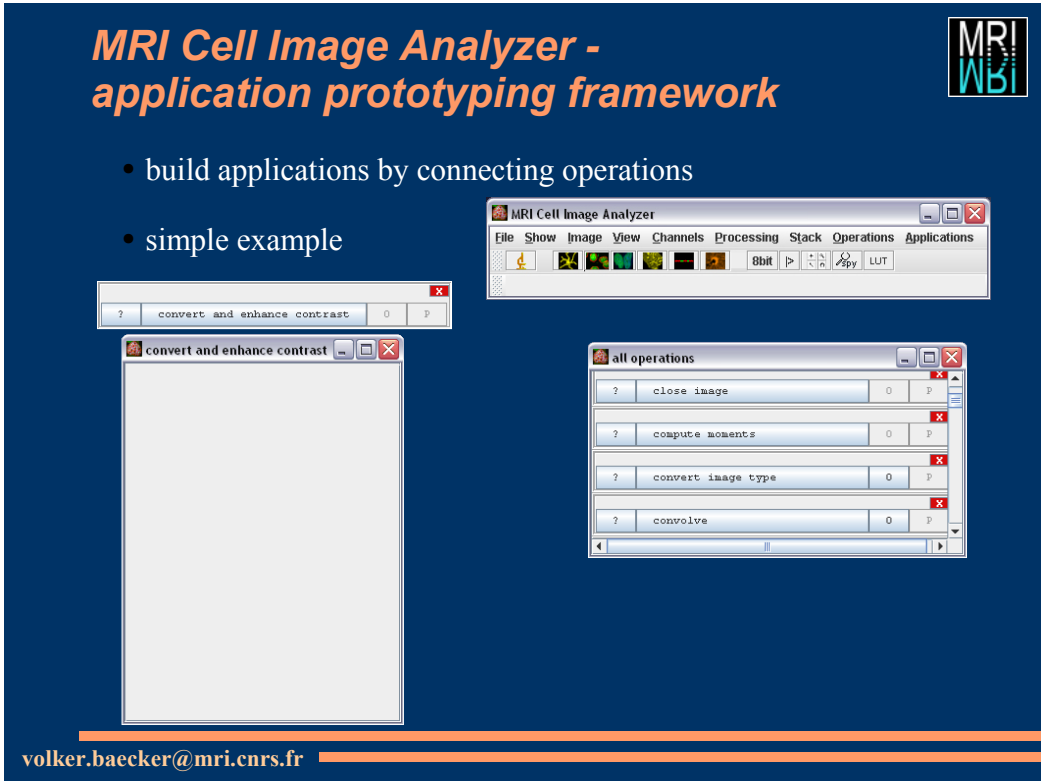


You get a tile representation of the new (still empty) application and a box representation.

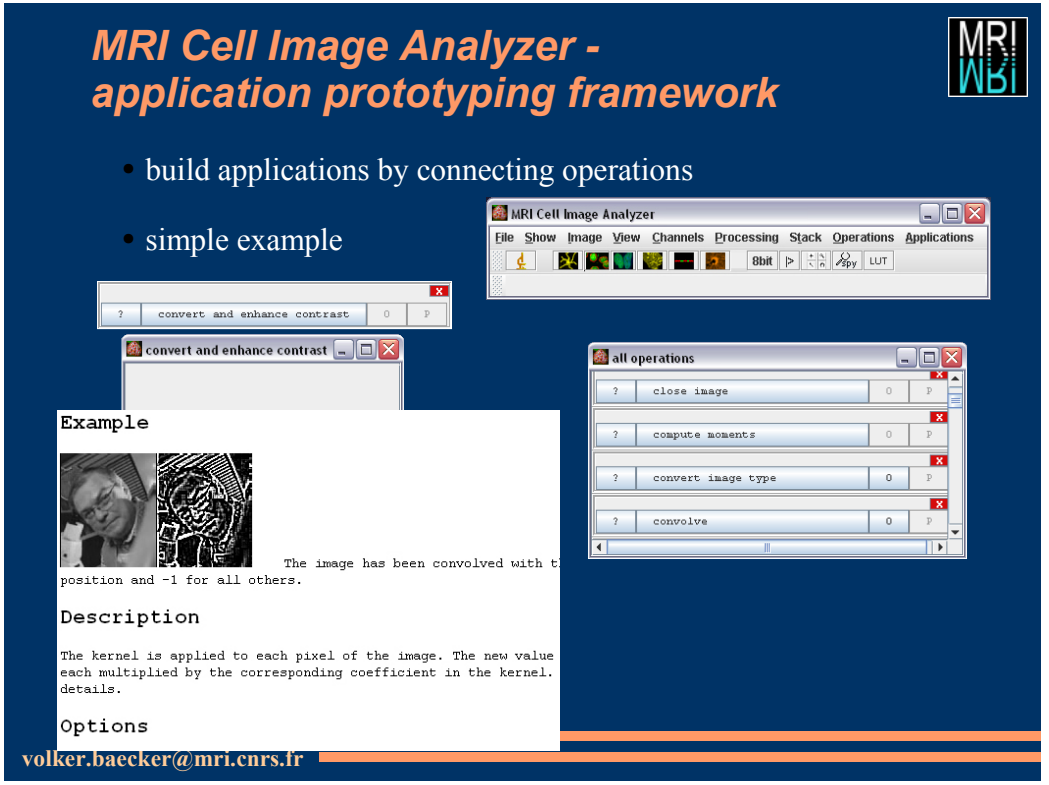
The application will be created by dropping operations into the box



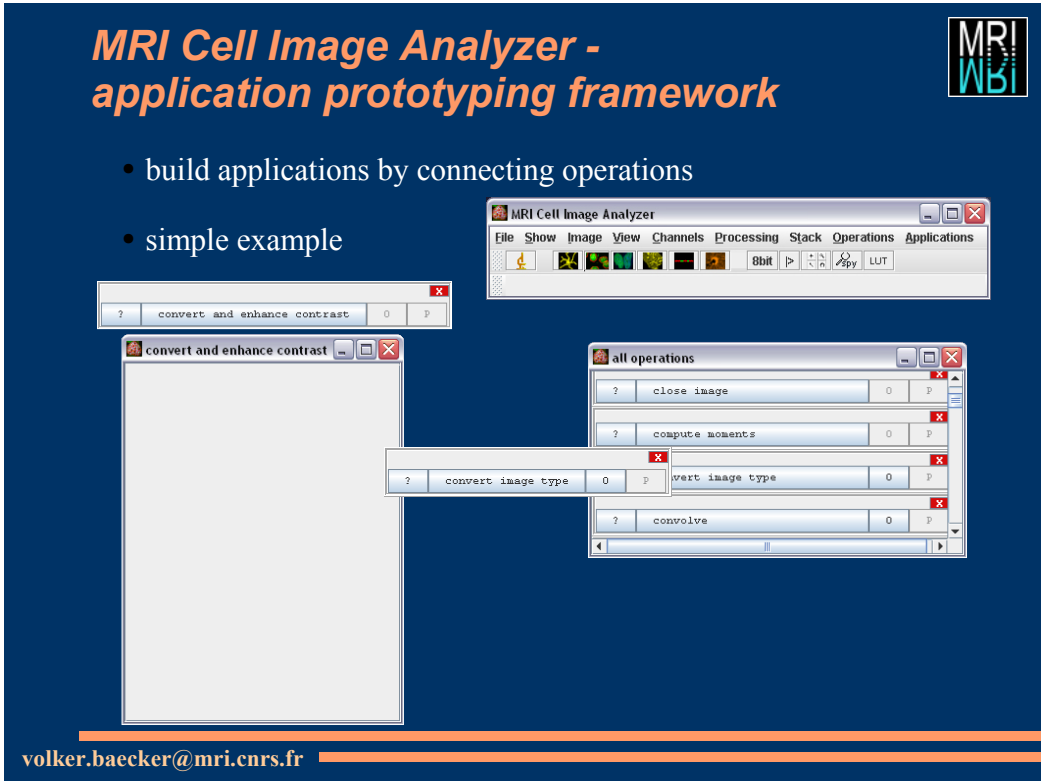
Go to the menu operations and open a collection of operations. Collections order operations thematically and the collection “all” contains all existing operations.



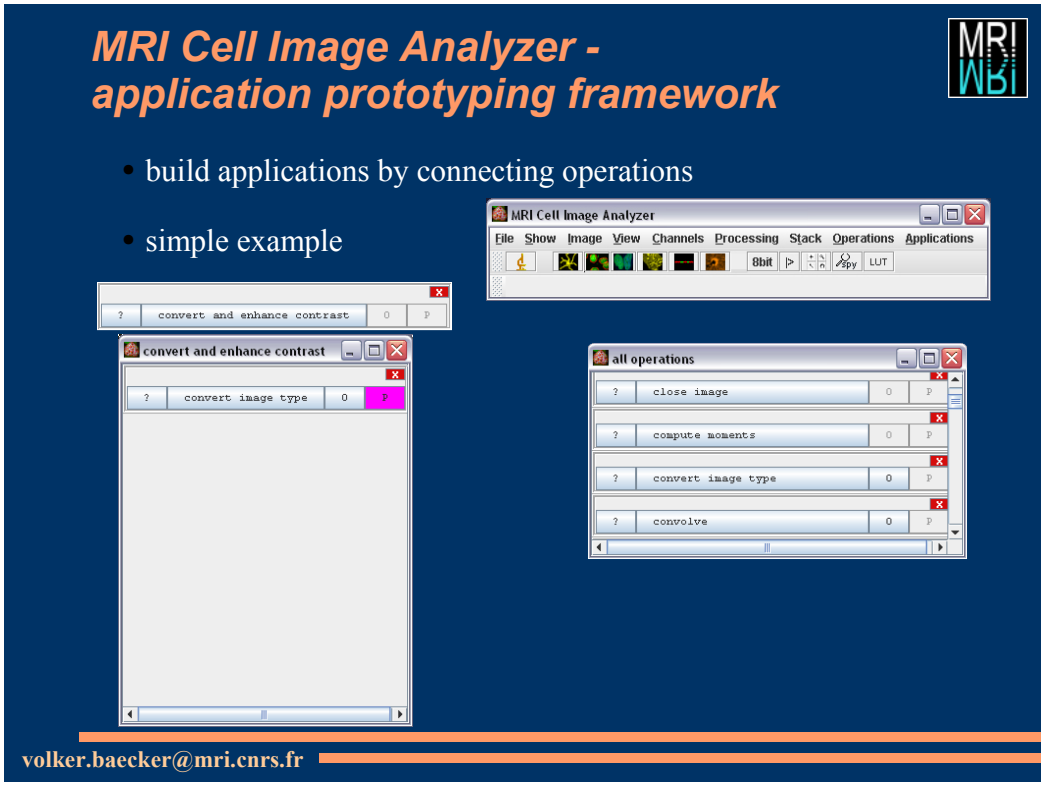
In the collection all we search the operation “convert image type”



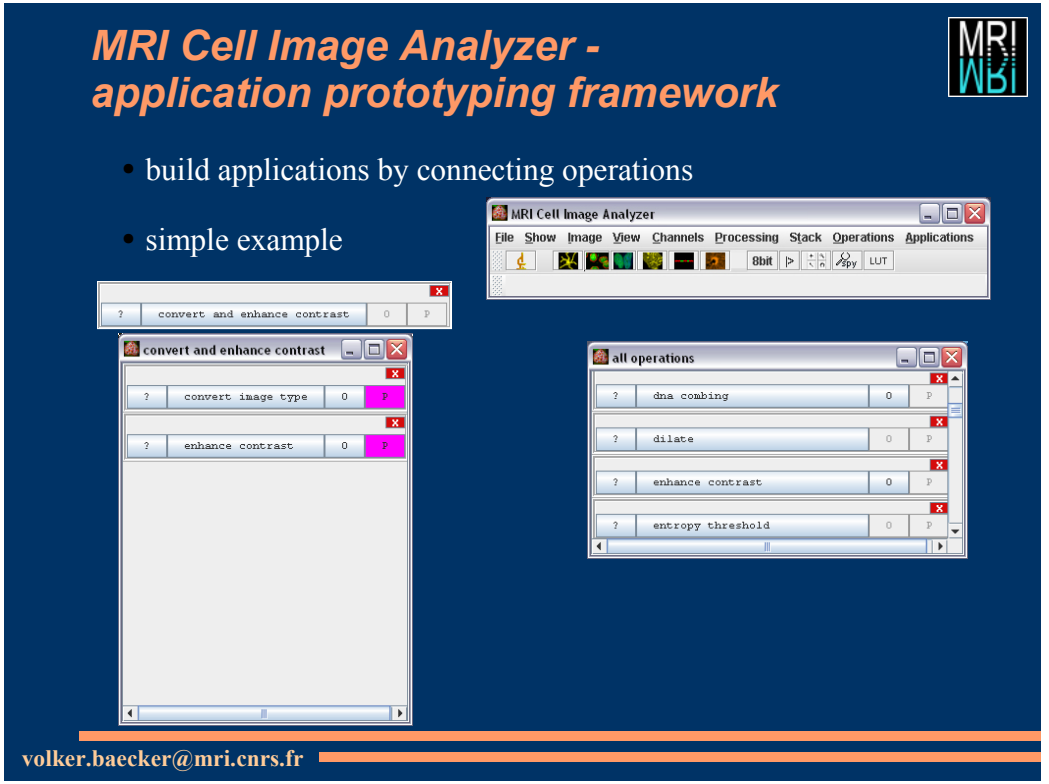
If you need information about an operation you can click on the questionmark. This opens the help text for the operation in your web browser.



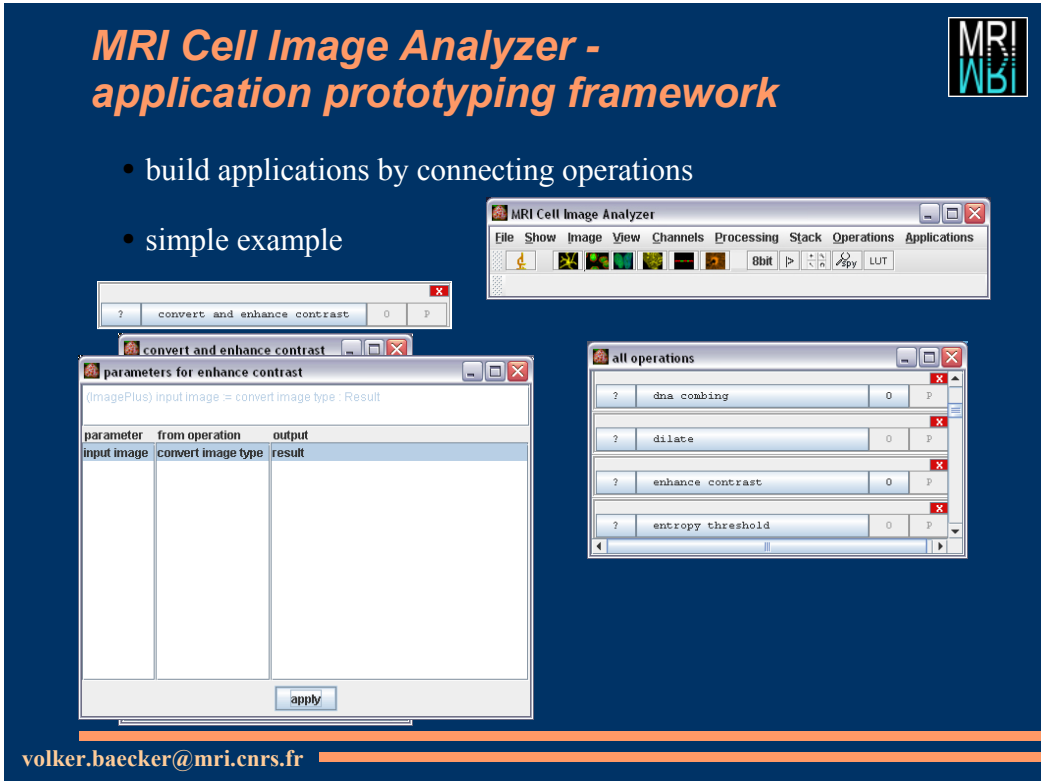
Dragging an operation from a collection, creates a copy of the operation.



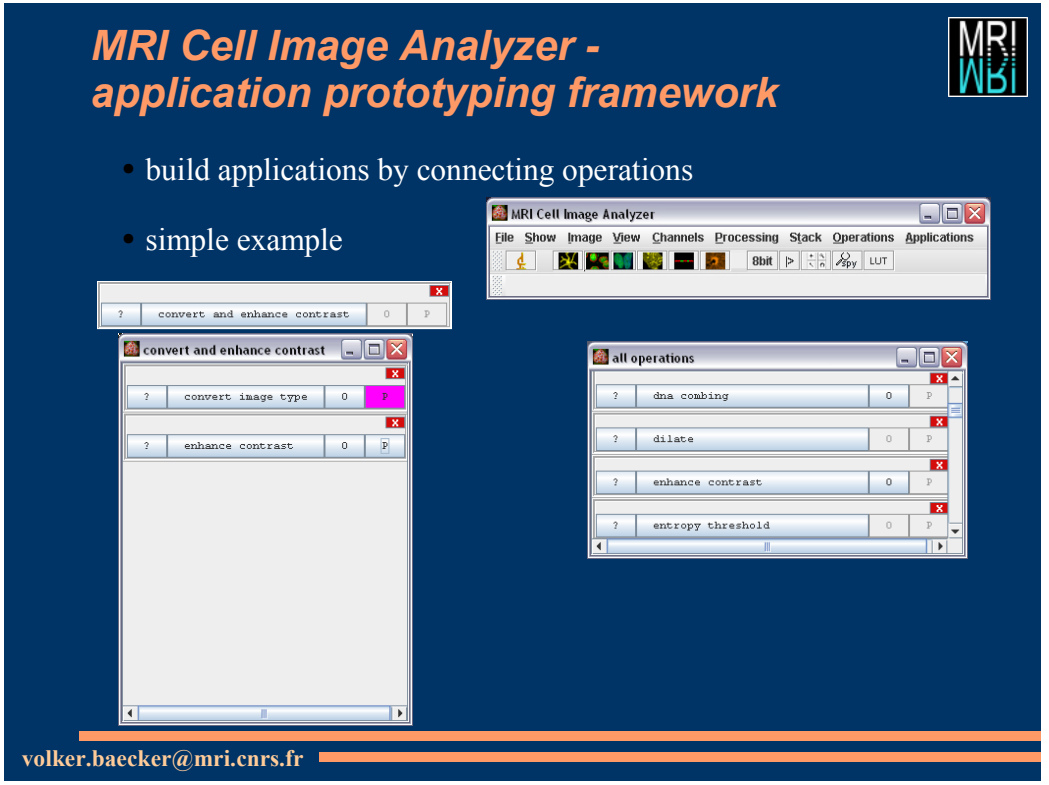
Drop the operation into the application box. The parameter button becomes red signaling that the operation needs an input parameter, that is the image to convert, in this case.



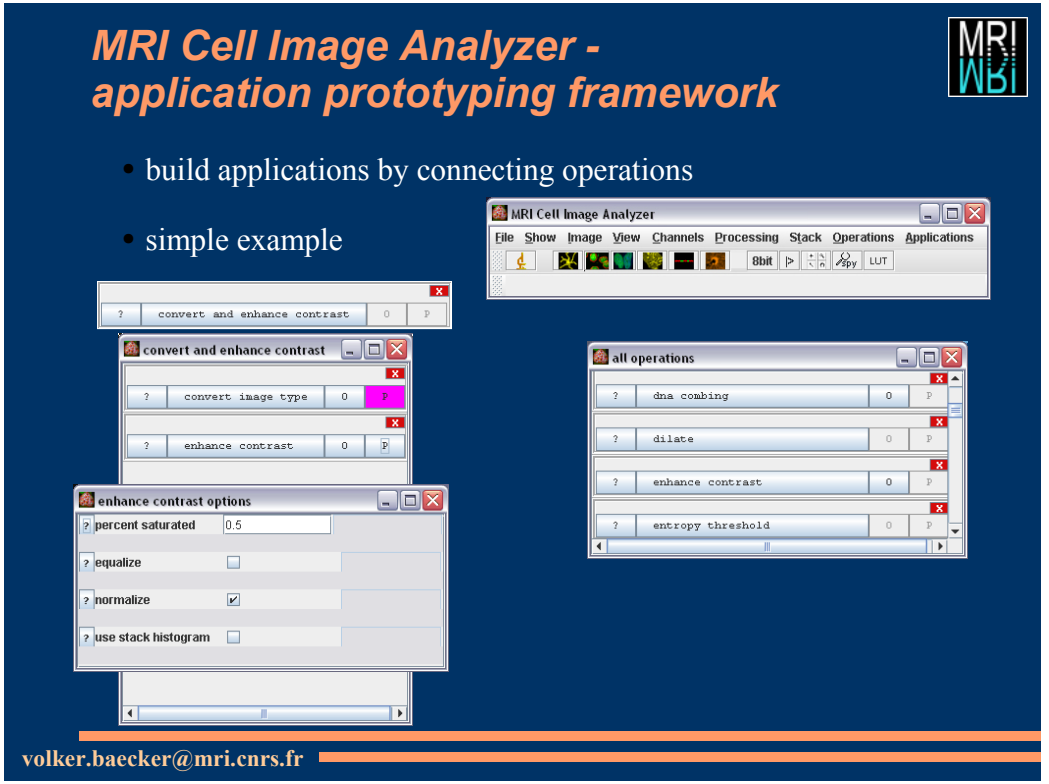
We add the operation “enhance contrast” in the same way and clock the “P” button to connect it with the first operation.



You get a list of all available inputs for the operation.

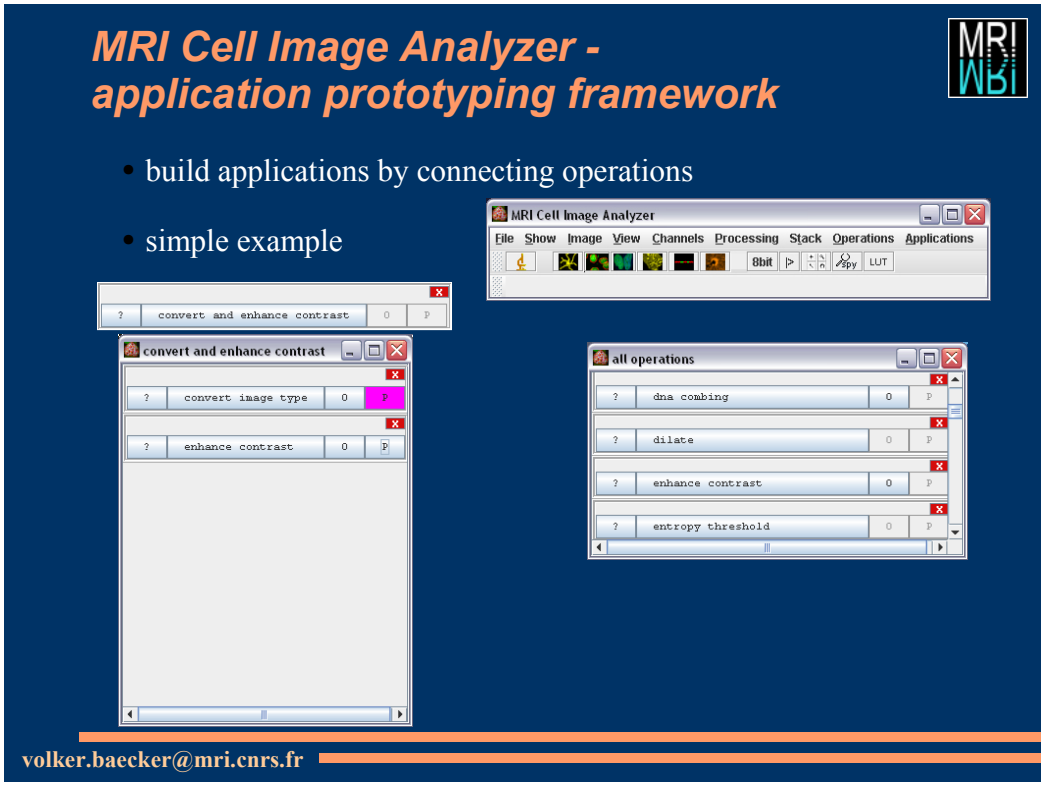


It is connected now.

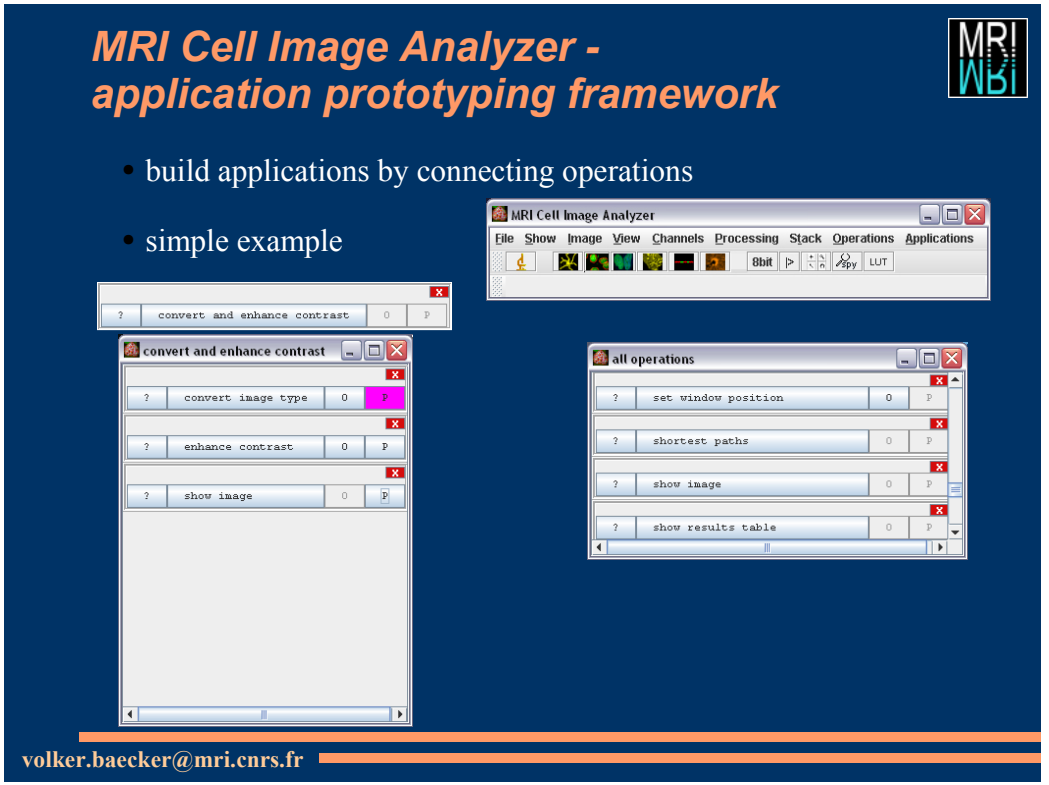


Use the options button to set the options of an operation.

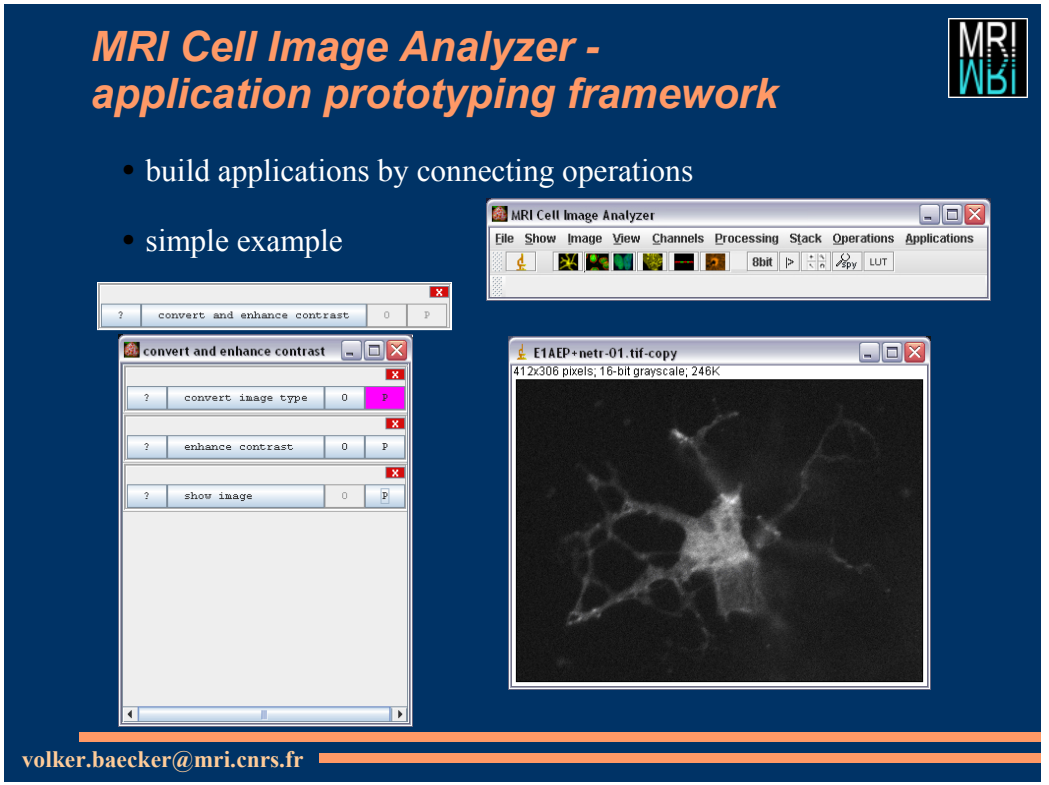
We choose the option “normalize” to do the contrast enhancement by a histgram stretch, allowing a maximum of 0.5% of all pixels to become saturated.



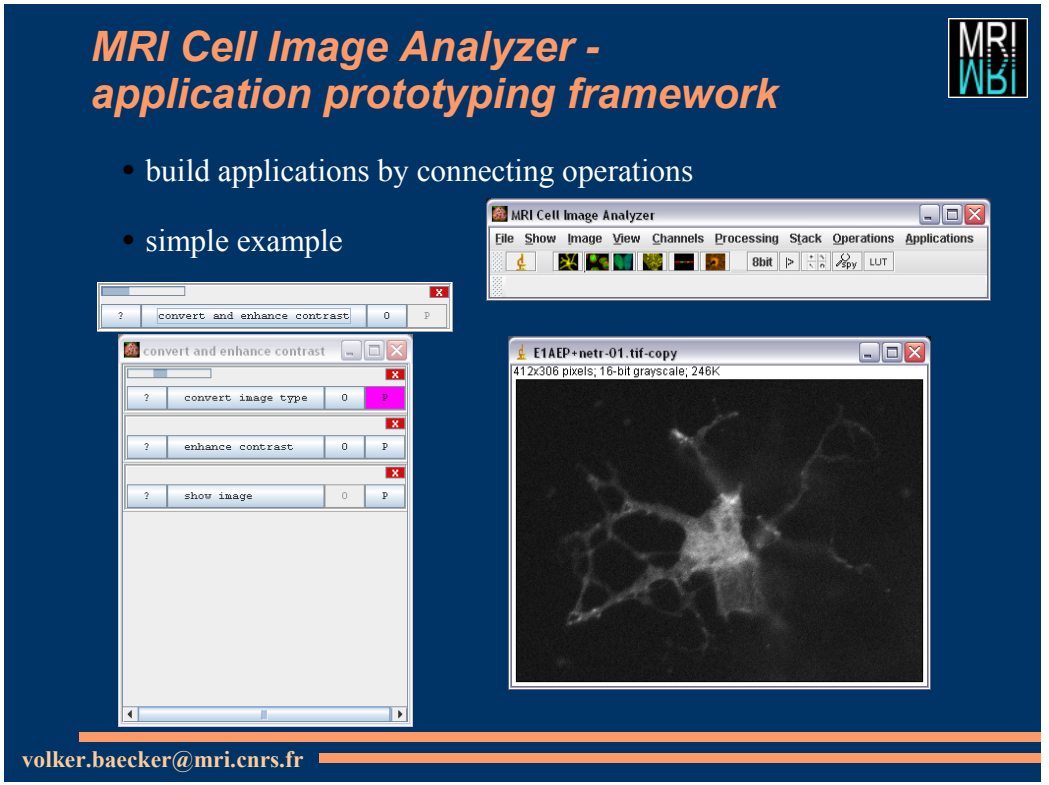
The application is finished now. However we want to see the result, as well.



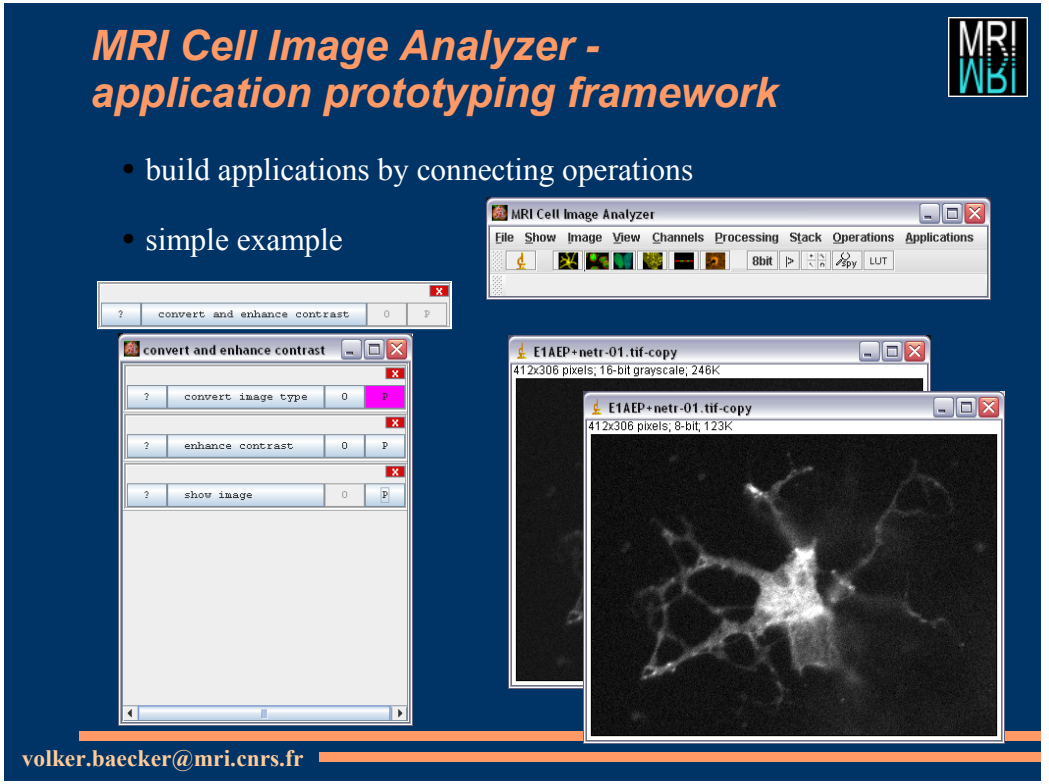
So we add the operation “show image” and connect it with the “enhance contrast” operation.



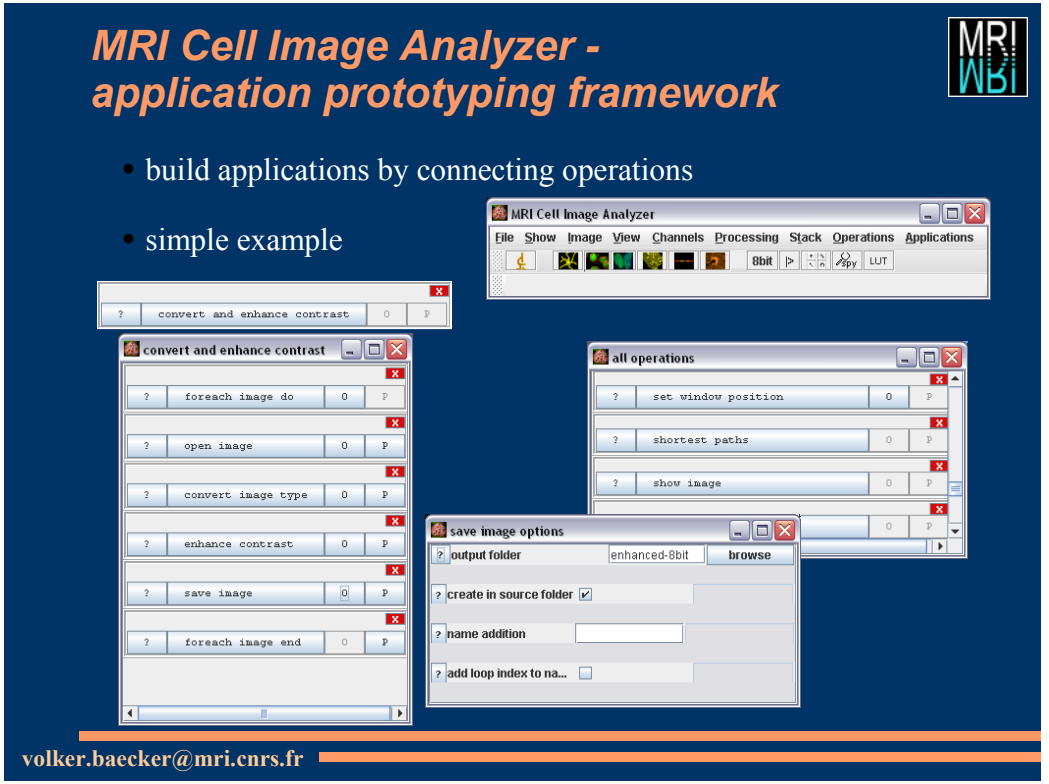
Let´s run the application now. We open an image and click on the central button of the application´s tile representation.



The application starts to run which is indicated by the progress bar of the application and by the progress bar of the current operation.



The application has finished and the result image is displayed.



Now this is all very fine, but I have got 1532 images to do the same thing with

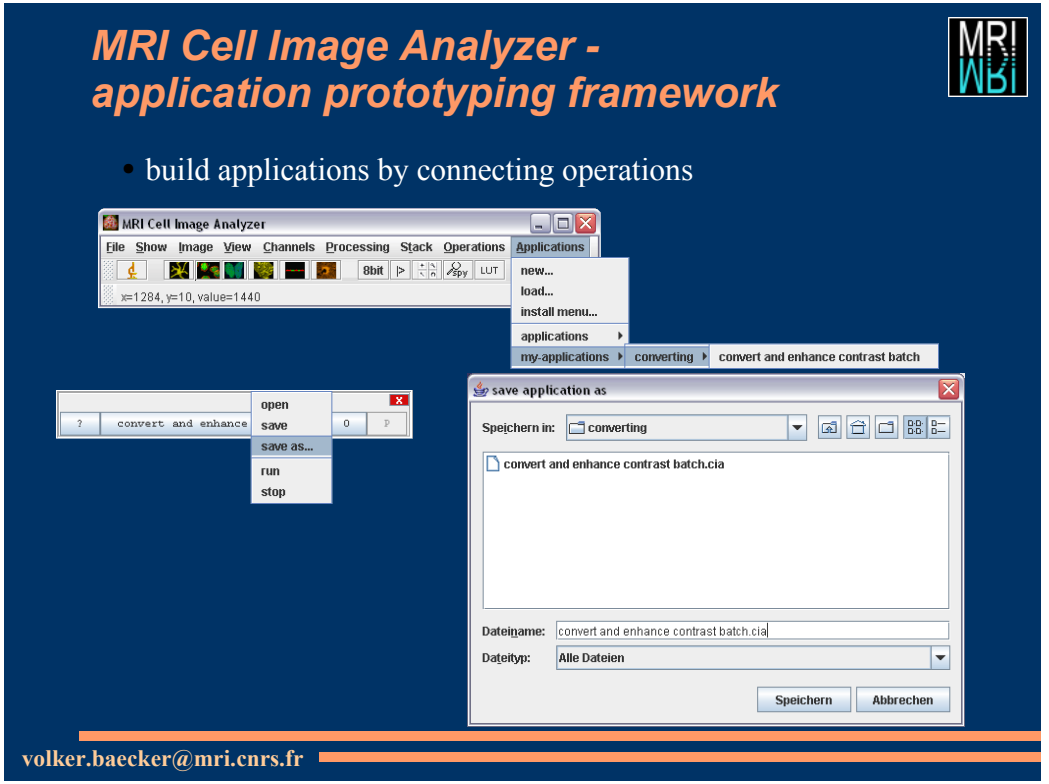
So I expand the application by adding a loop to read in all my images one after the other and to save the results.

In the save operation, a place for the result images can be configured.

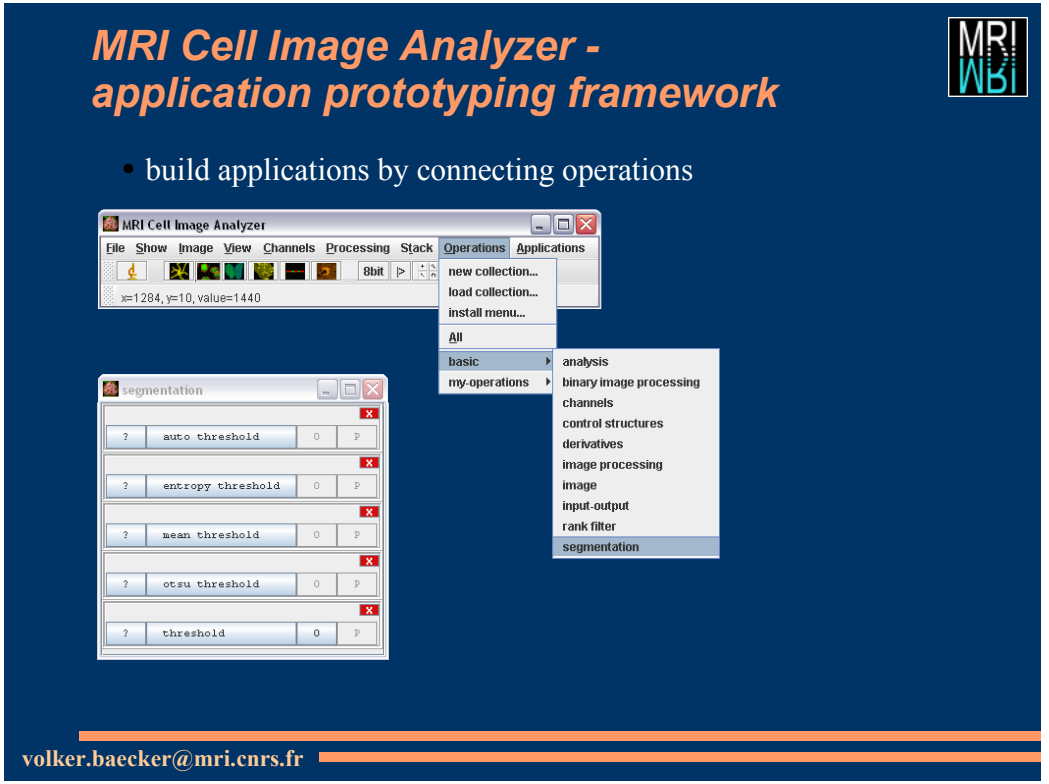
In this case they will be saved in the folder enhanced-8bit that will be created as a subfolder of each source folder.

Fine, I can convert all my images now.

Now I want to save my application, in case I´ll need it in the future.

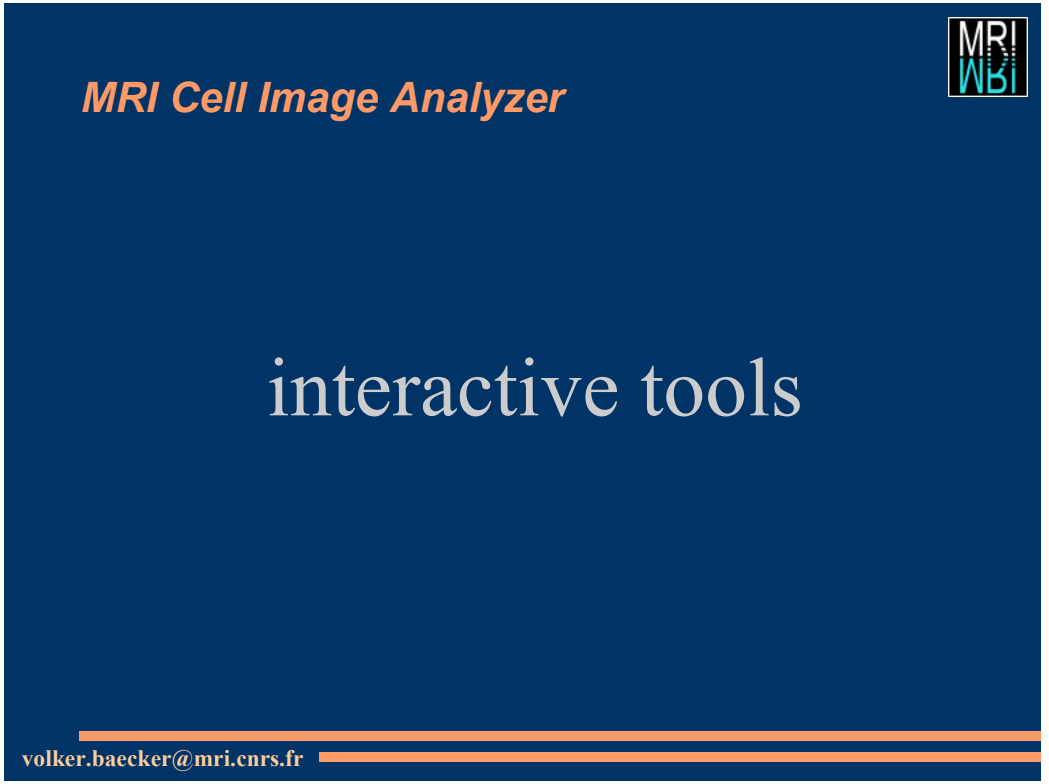


Right clicking on the application's tile opens a context menu, that allows to save the application. If it is saved in the applications folder it becomes immediately available in the applications menu. If it is saved elsewhere use “install menu” to make it available from the applications menu.

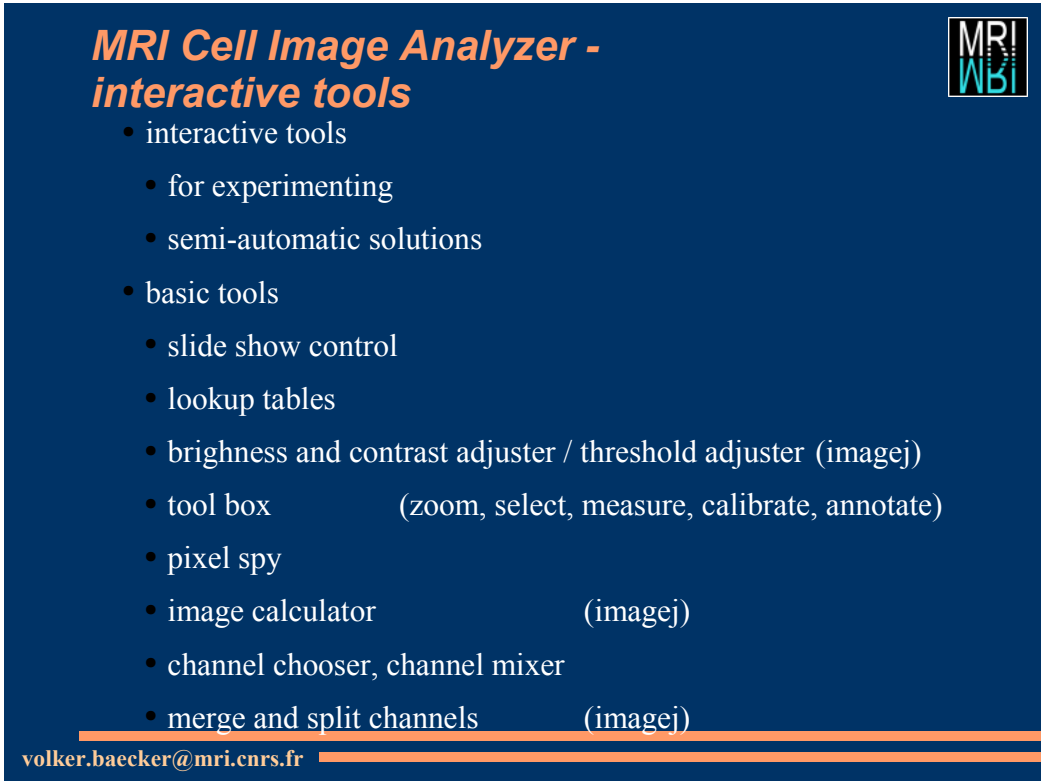


In the same way new collections of operations can be created, saved and made available in the menu.

You saw how to create new applications from existing operations and how to apply them to a list of images.

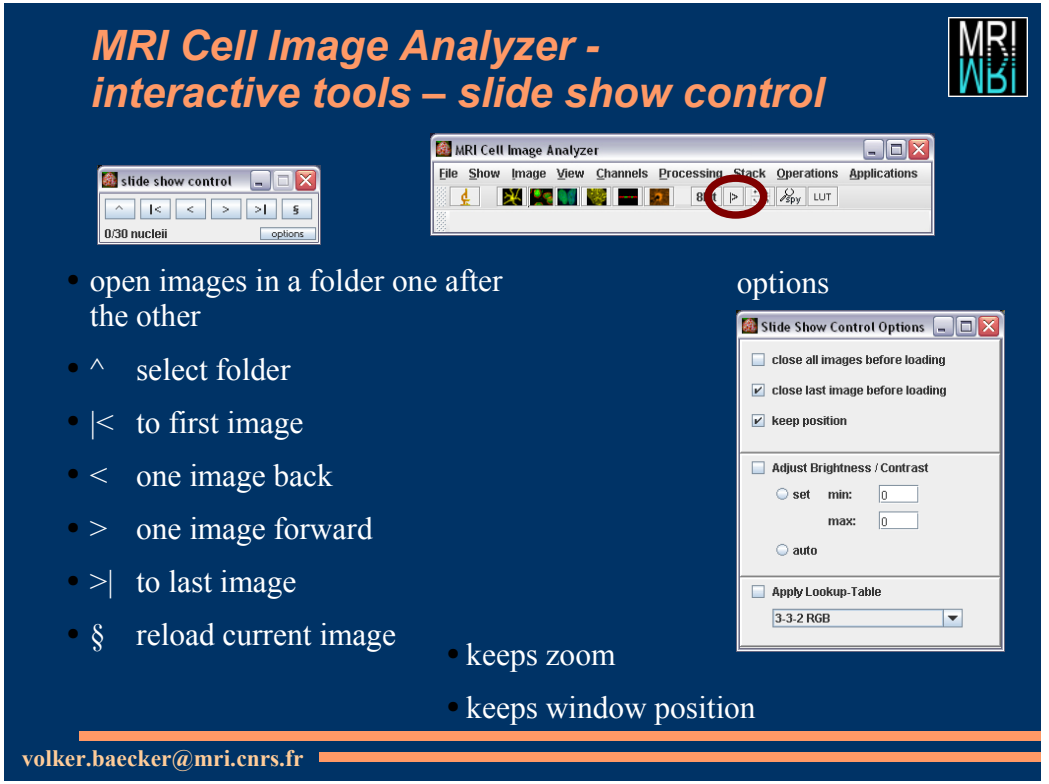


I want now to give a short overview over the interactive tools available in the environment



Although the goal is the automation of tasks, interactive tool are needed when searching a solution to a problem and in semi-automatic applications where the user has to adjust things before the application continues.

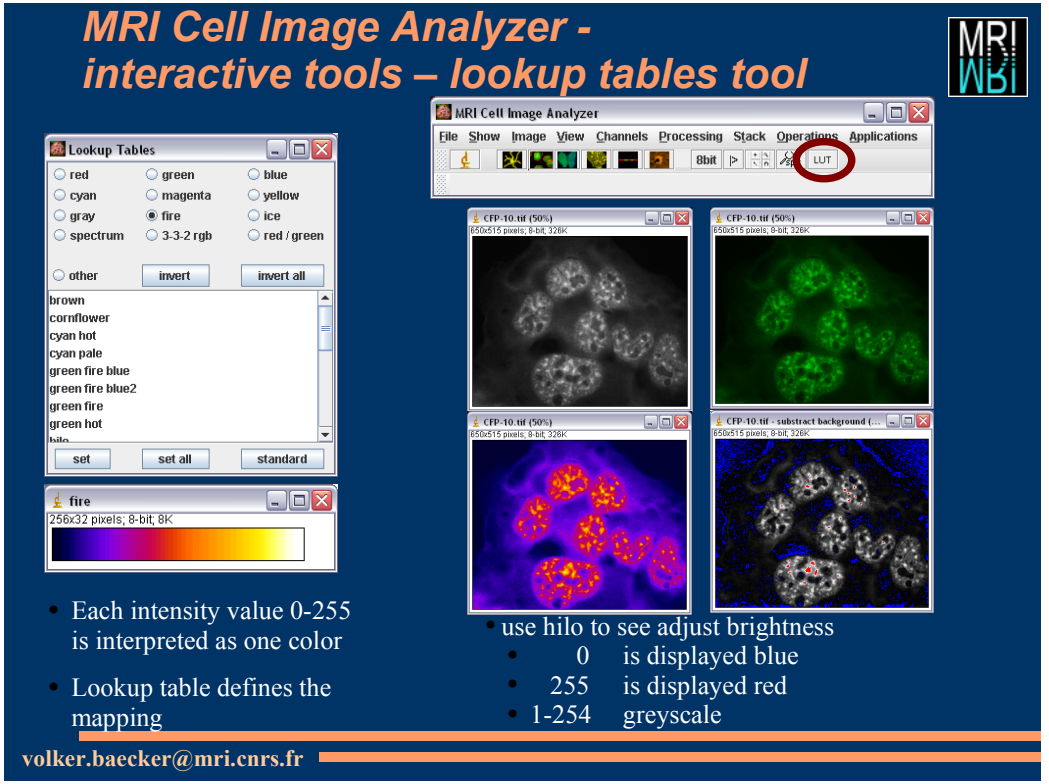
Some of the tools were already available in ImageJ. Others were added by me because they were needed for an application.



The slide show control allows to open all images in a folder one after the other. The next image will open in the same place as the last one and have the same zoom.

You can tell the slide show control to adjust the brightness and contrast when an image is opened either automatically or to some fixed values.

You have the option to automatically apply a lookup table when an image is opened.



The lookup table tool allows you to show a greyscale image with colors.

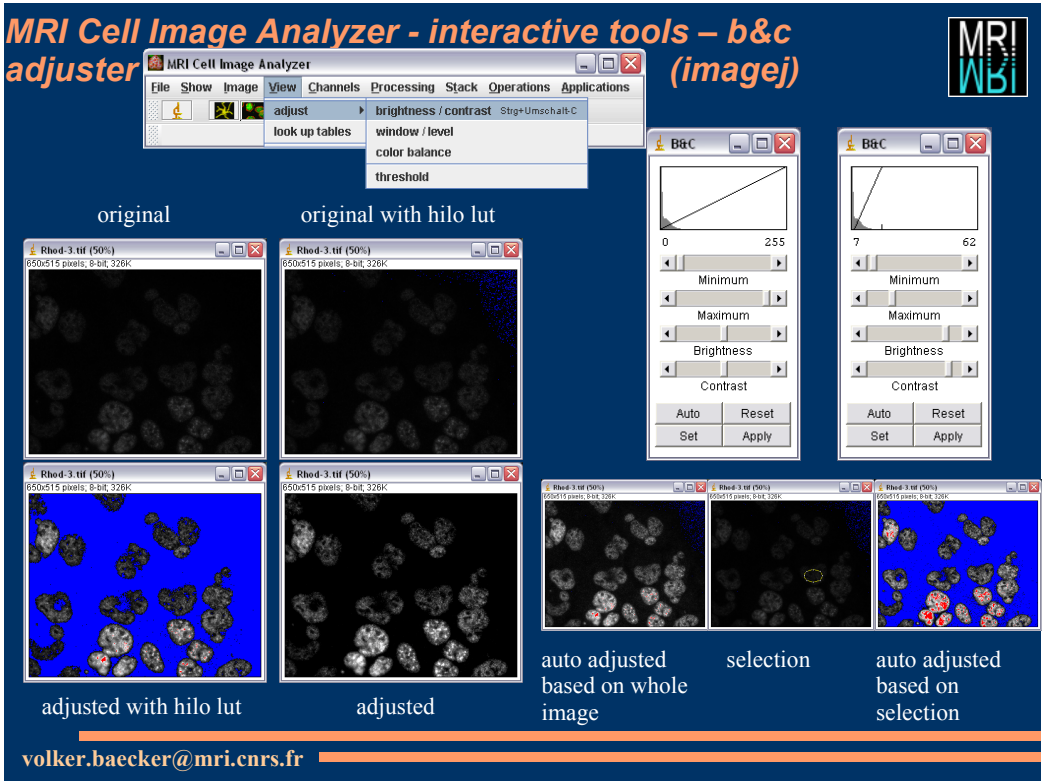
These can reflect the real wavelength or they can be choosen arbitrary to allow you to recognice better the details you want to see.

How does it work?

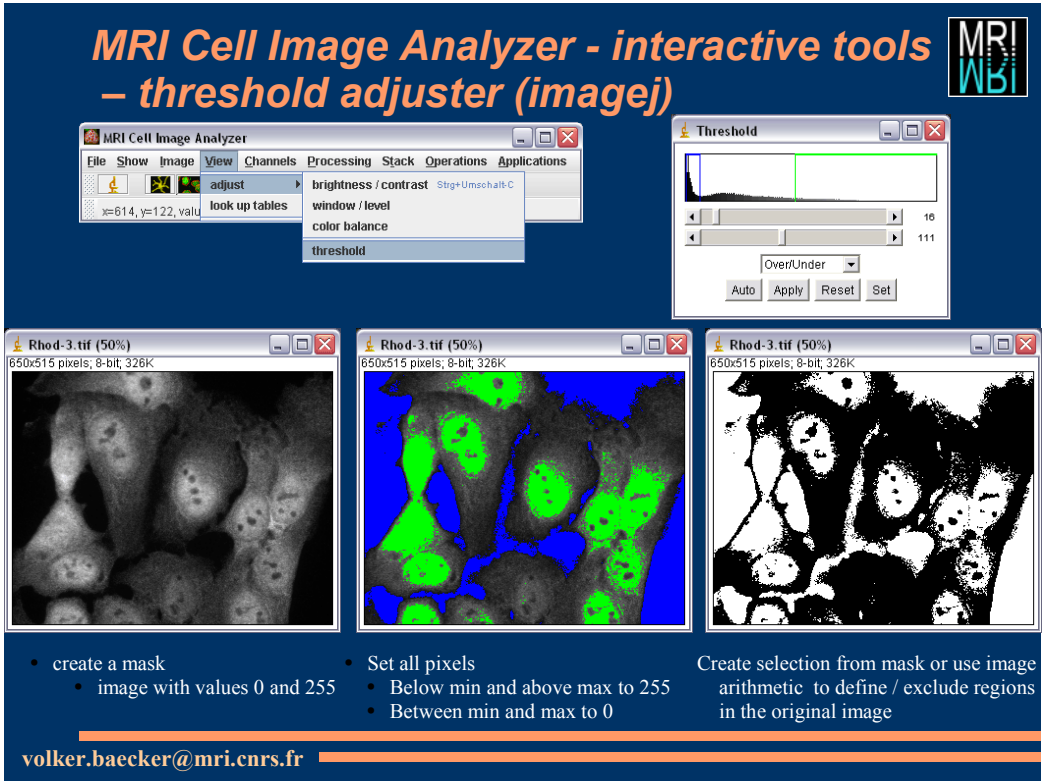
An 8 bit image contains 256 values from 0 to 255. With a lookup table each value is mapped to a color and each pixel in the image that has this intensity value is shown in the associated color.

The lookup table “hilo” is especially useful. It displays 0 intensities in blue. Maximum intensities in red and everything between in the normal greyscale.

Use it when you adjust brightness / contrast.



With the brightness and contrast adjuster you can make adjustments manually or use the auto button. If you want the auto-adjustment to be based on a selection, make a selection before pressing auto.

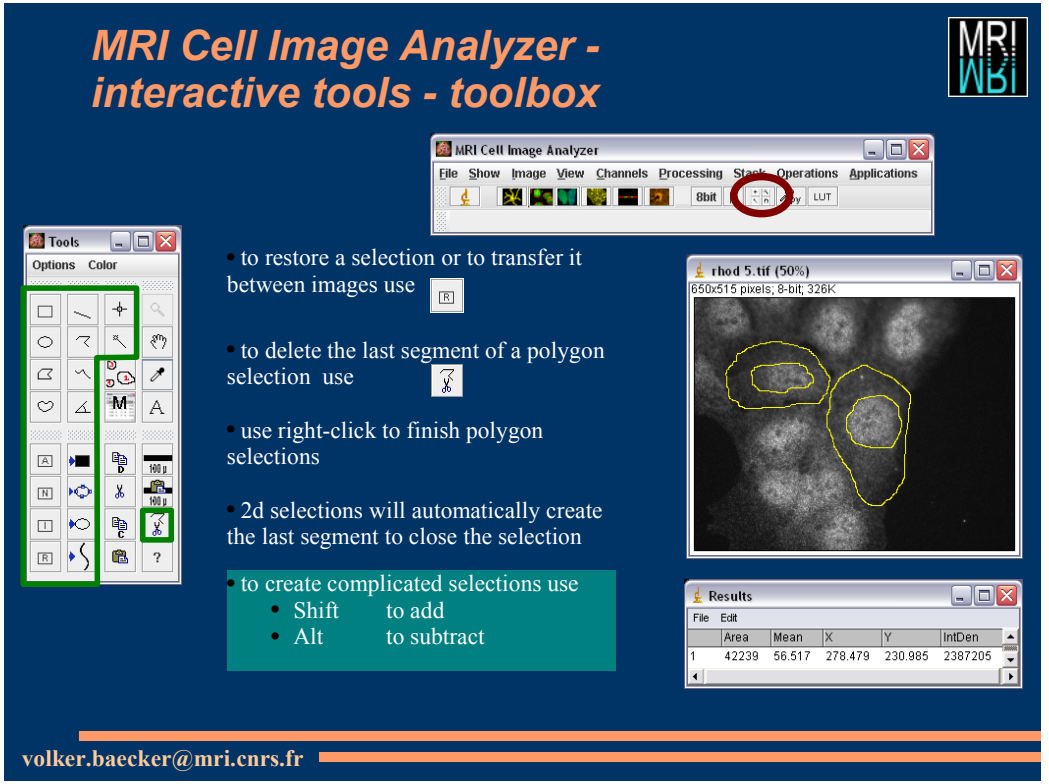


Use the threshold adjuster to create a mask seperating background and objects.

You choose the min and max values.

All values below min and above max will be set to 0. All values between min and max will be set to 255. Leaving you with the mask, that is an image that contains only two intensities.

You can use a magic wand tool to create a selection from a mask. You can then transfer the selection to the original image.



To give quick access to important commands I created the toolbox. Most of the commands concern the creation of selections.

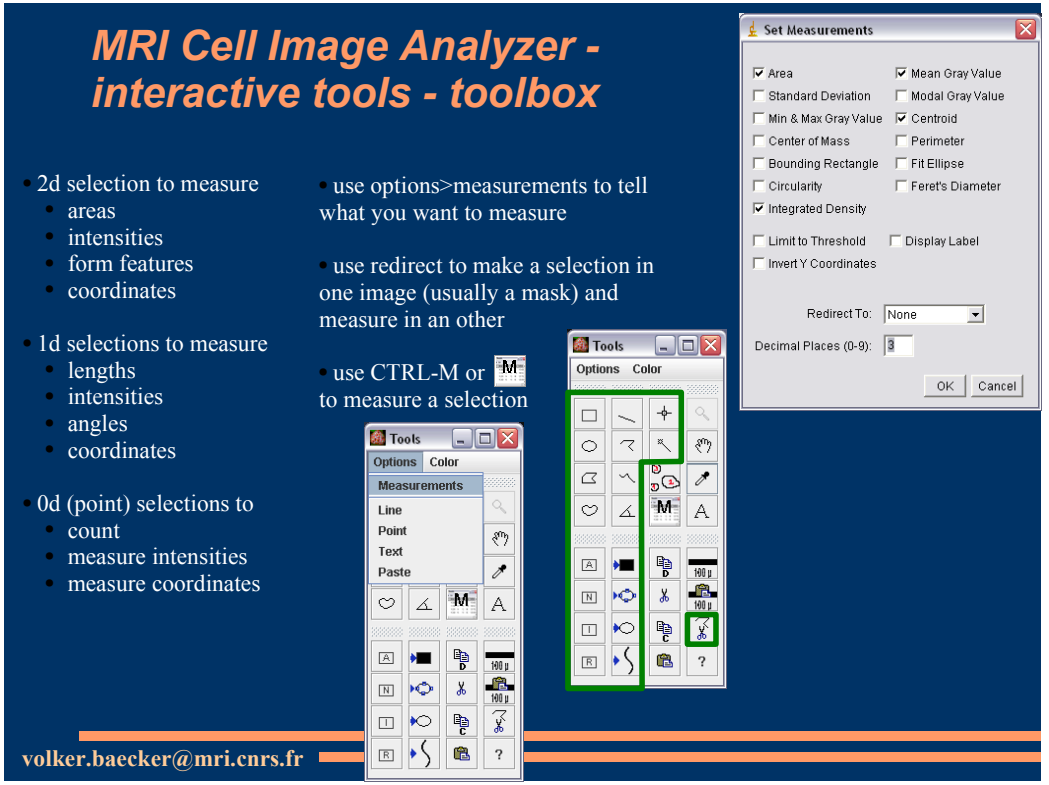
Use the R button to restore a selection or to transfer it to an other image.

A command I added is the to delete the last segment of a polygon selection. Allowing to correct errors without re-selecting everything

When you make an area selection the area will be closed automatically.

You can create complicated selections by subtracting from and adding to an existing selection.

The selection in the example contains the areas around the two nuclei.



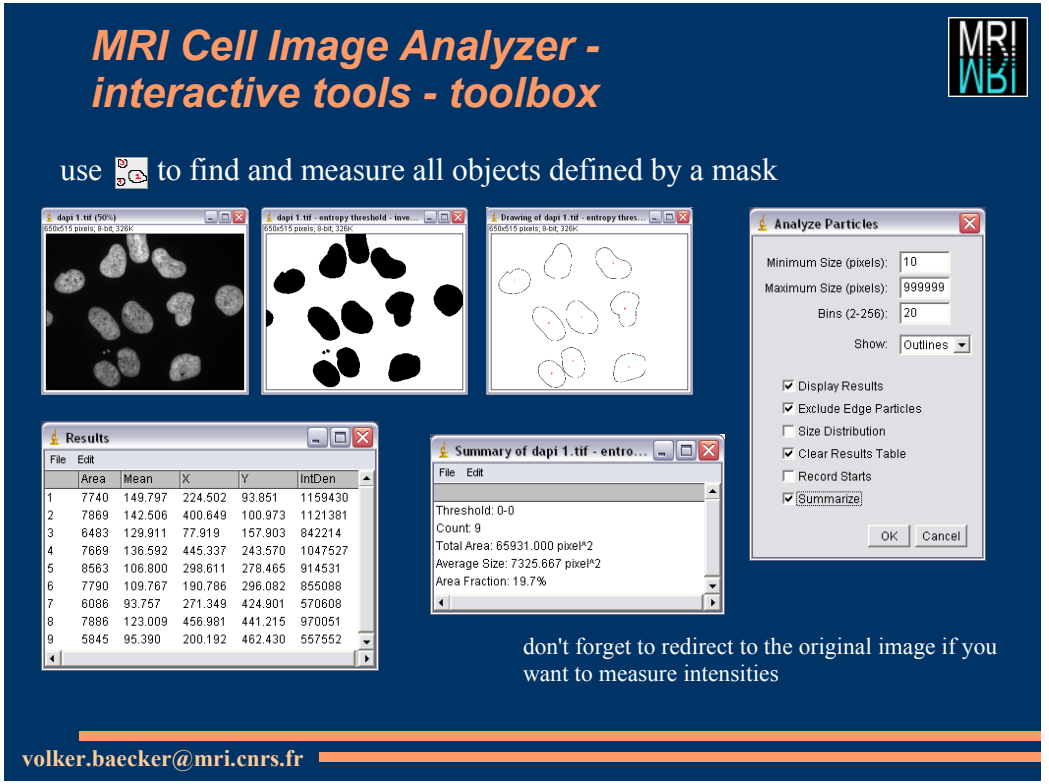
With area selections you can measure surfaces, intensities and form features, like for example circularity.
With line selections you can measure lengths and angles
You can use point selections to count objects.

In the options-measurements menu you can select what measurements will be made.

Select redirect if your selection is in one image and you want to measure in an other image.

Use the “M” button to measure a selected region.

Results are shown in a table that can be saved and imported into a spread sheet program.

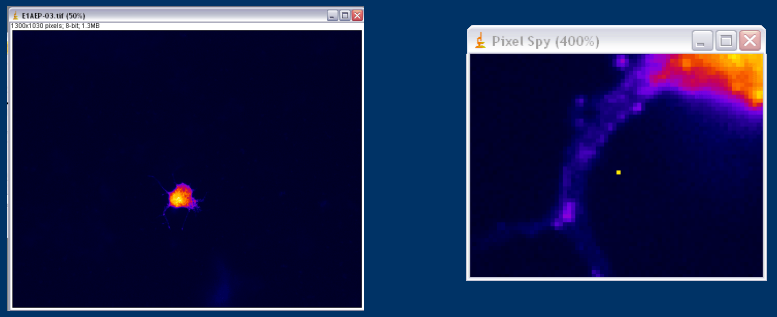


Use the find objects button to measure all objects defined by a mask.

You can limit the objects taken into account by restricting the min and max size.

Again you can redirect the measurement to another image, usually the original image the mask was created from.

MRI Cell Image Analyzer - interactive tools – pixel spy



a magnifying glass that shows the region under the mouse pointer

- you can change the zoom and window size of the pixel spy

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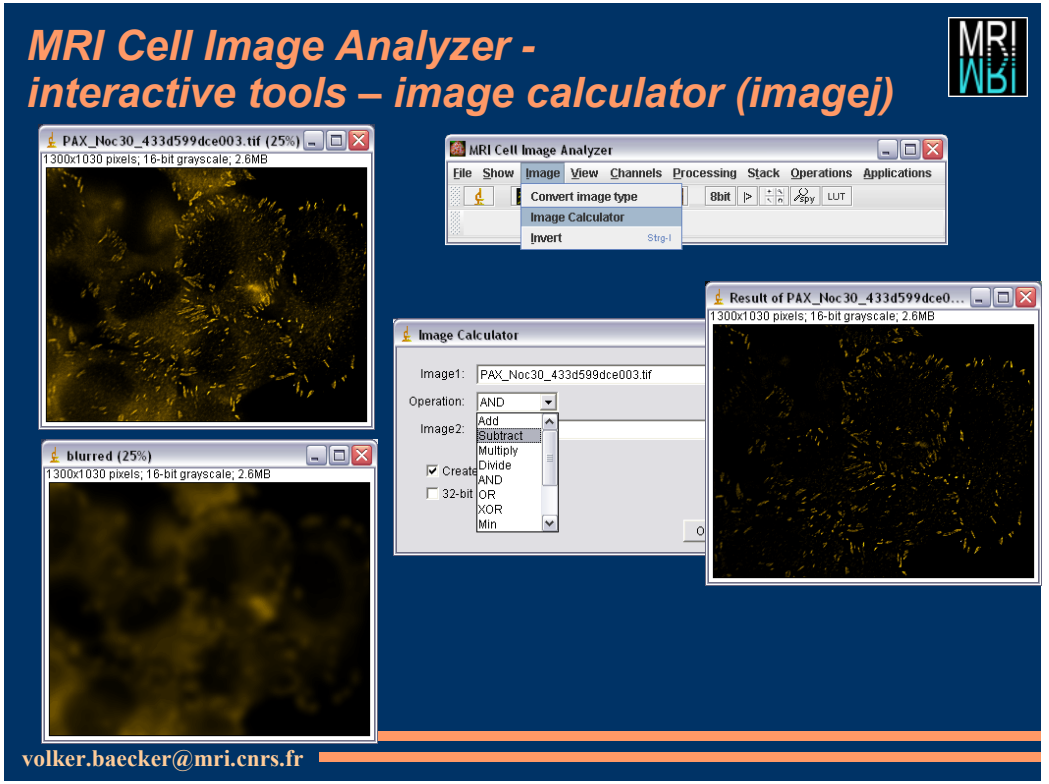
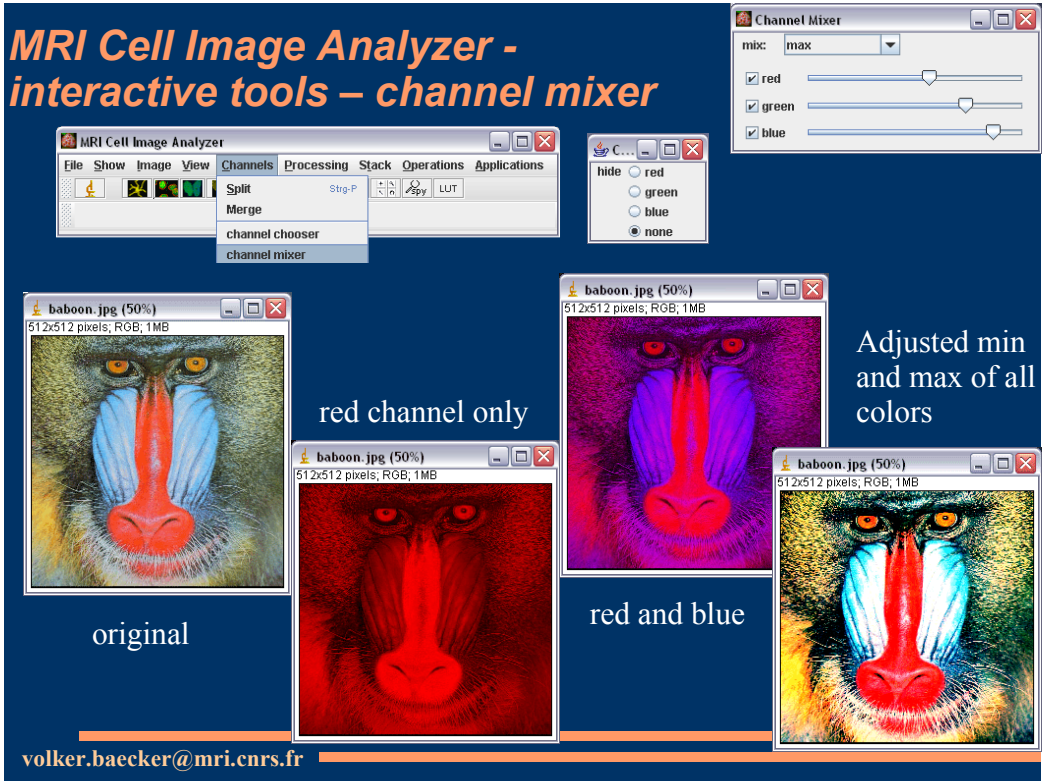
The pixel spy shows the a magnified area around the mouse pointer.



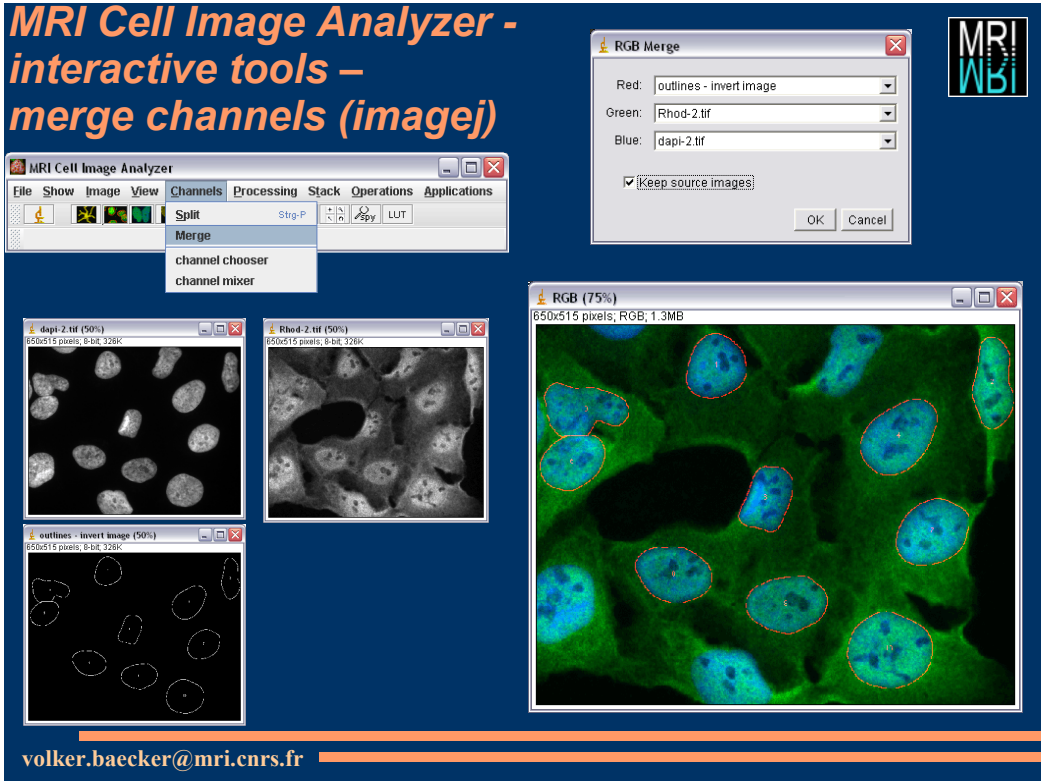
The image calculator takes two images and computes a third one by applying the selected operation pixel by pixel.

In the example a blurred version of the image is subtracted from the original version to leave only the small structures of interest.



In color images select the channels to be displayed or adjust the channels independently so that details in one channel are not hidden by the other channels.

ImageJ has a tool to do this, but you either have to change the intensities in the image (not just the displayed intensities) after each adjustment or the previous adjust will be lost.



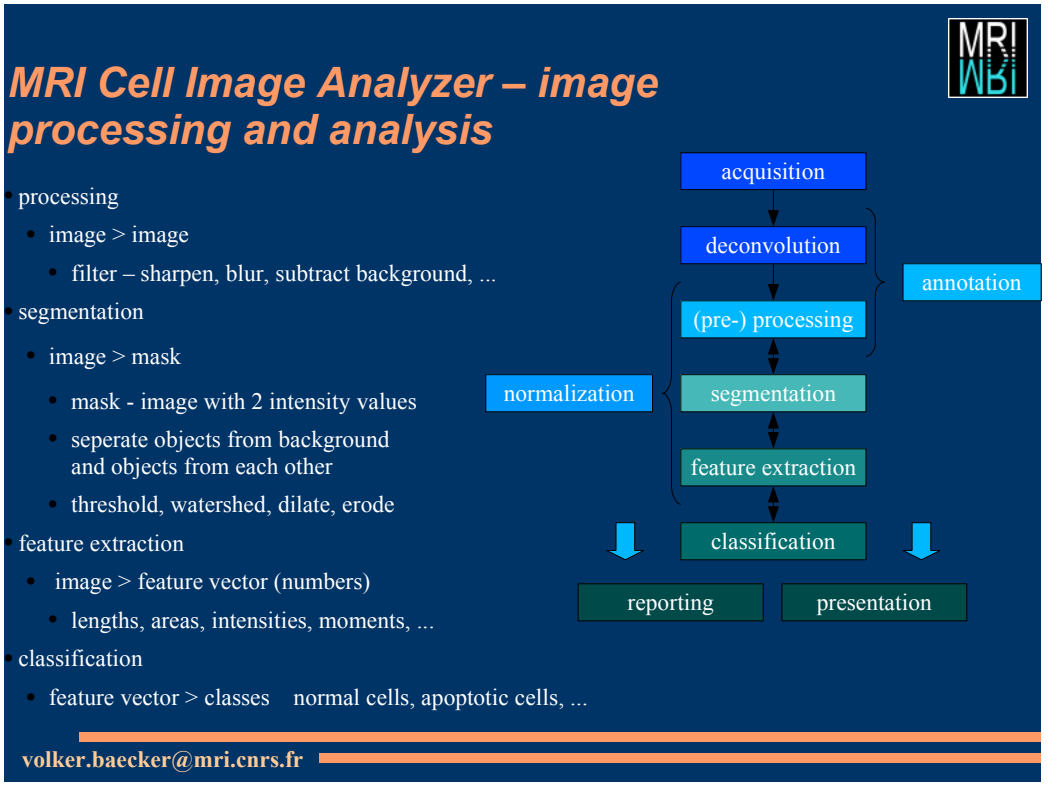
Merge channels to create an rgb image from greyscale images.

You might want to do this for example to show where the stained objects in your cells are.

Another application is to show which objects have been found by an application and to see which measurements belong to which object. So you can verify the result of the application.

You have seen the available tools now let us have a look at what image processing and analysis means.

And go through the process with the help of an example.



The first step is the acquisition of images, done with the help of microscopes, ccd cameras, etc.

The next step is deconvolution to get remedy the distortions created by the imaging process.

The aim is either analysis, that means extracting data from the image that is not explicitly available, or presentation for example for a publication.

Processing creates an enhanced image from the input image, for example by applying filters. It may be enhanced in respect to the following analysis steps or in respect to presentation.

One of the most important steps is the segmentation. Segmentation divides the objects in the image from the background, and eventually the object from each other.

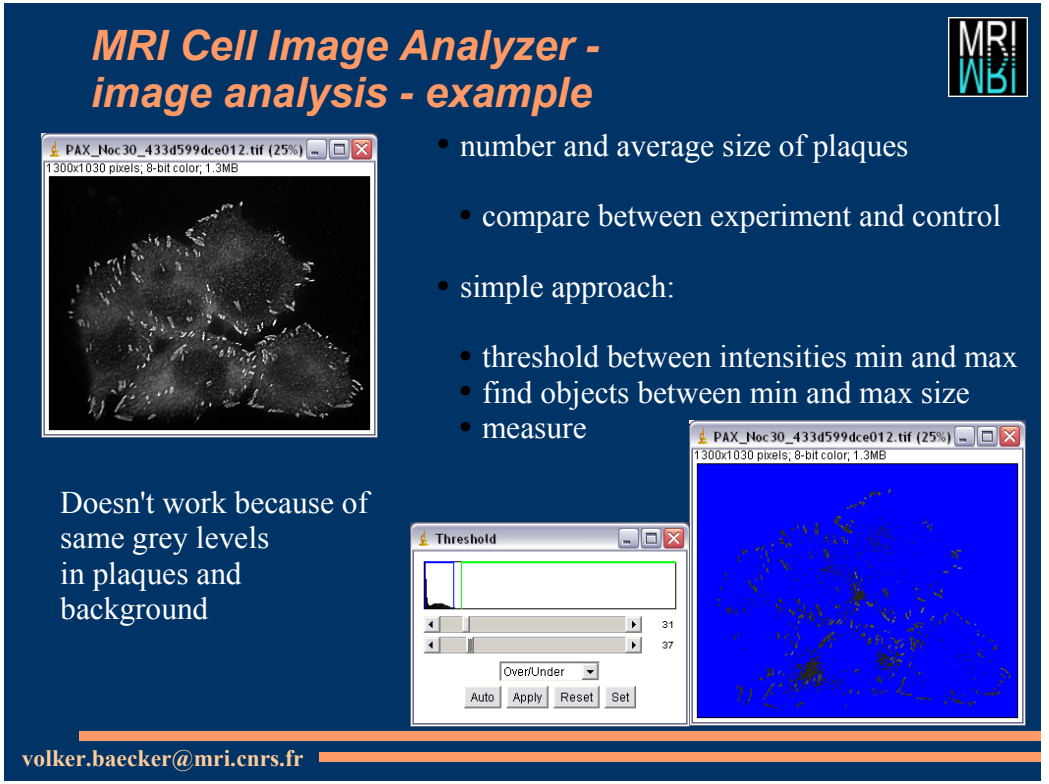
After segmentation features of the objects can be extracted. Either because they are of interest themselves or as the input for a classification step.

Classification divides the objects in the image into categories, for example normal and apoptotic cells

To do successful classification a normalization might be necessary, for example to make the features independent of the objects size, position or rotation.

You might want to annotate images, for example with the time passed in a time series or to point to regions or events in the image.

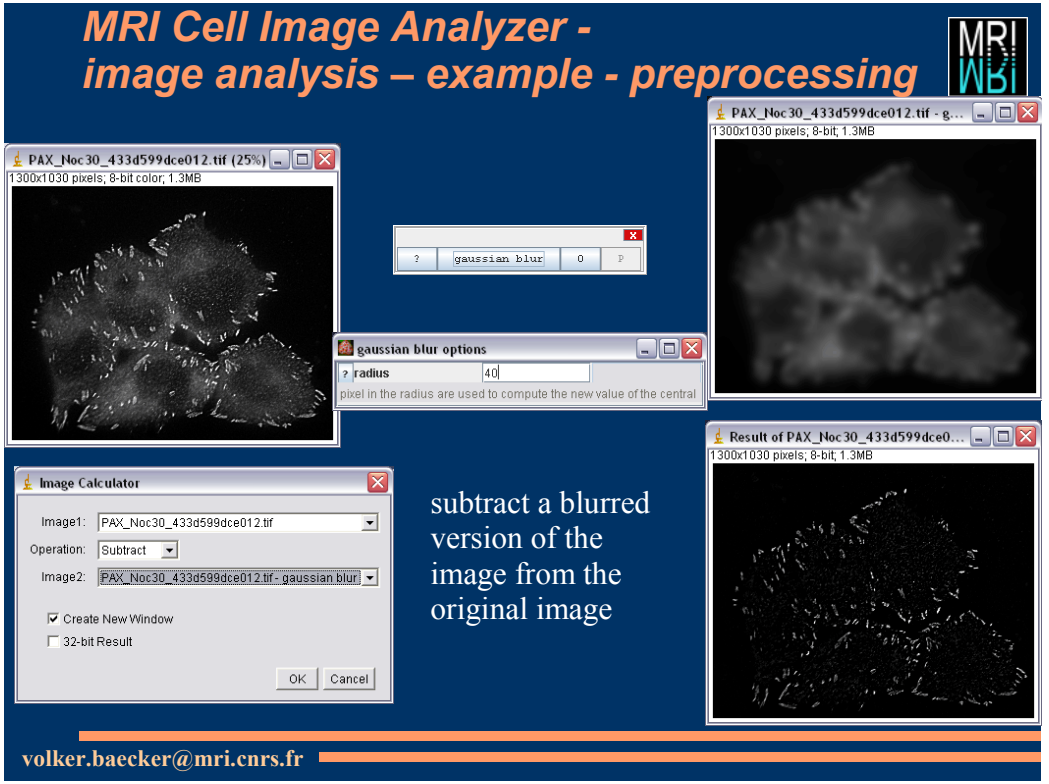
The result will be either a report of the extacted data or the processed image for presentation.



The task is to measure the number and size of the plaques in the image.

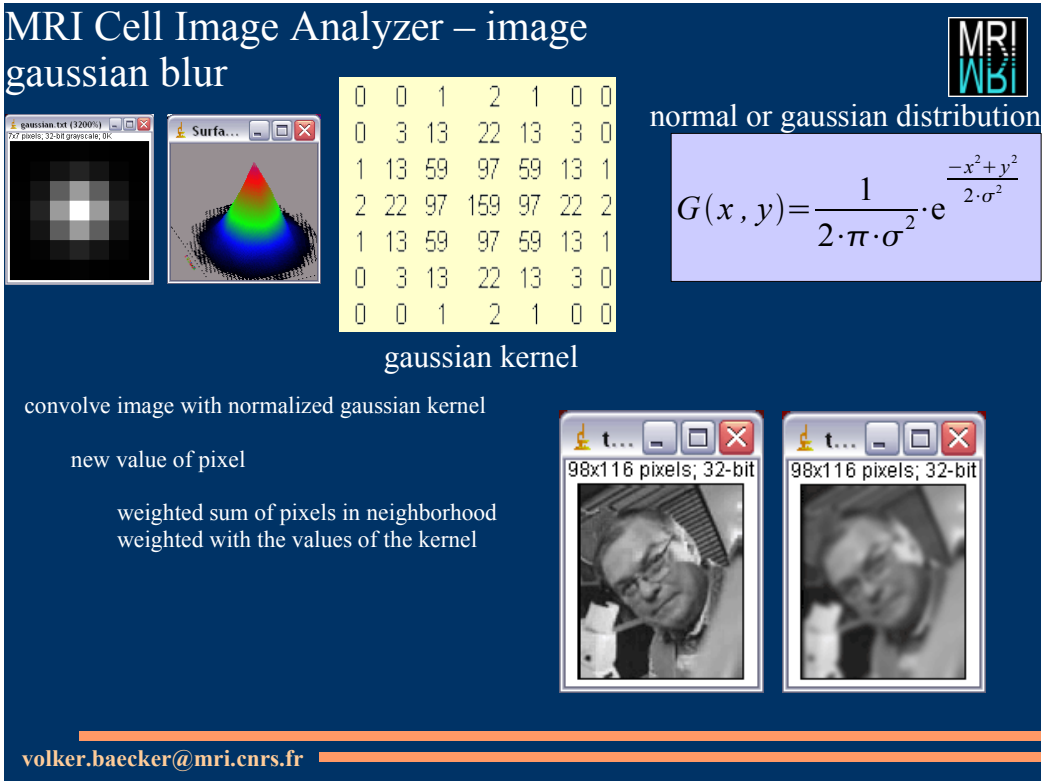
The hypothesis is that the experiment changes their number and size. So their number and seizes in images from the experiment and in images from the control shall be compared.

The simple approach to get them by giving an intensity and size range doesn´t work, because the background contains the same greylevels as the plaques.



We use a gaussian filter with a big radius to blur the image. In the blurred version the plaques have disappeared. Subtracting the blurred version from the original leaves us with the plaques and some background noise only.

However these can now be distinguished by their size.



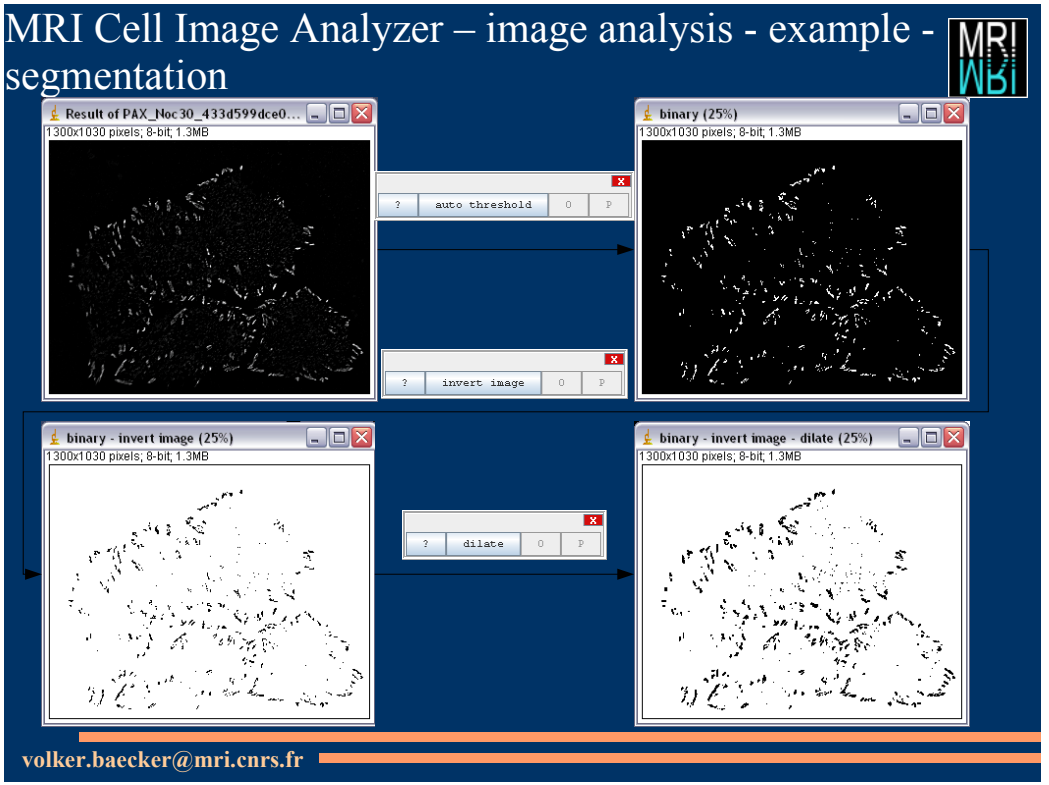
How does the gaussian blur work?

A matrix with values according to a gaussian or normal distribution is calculated. The matrix is called the kernel of the filter.

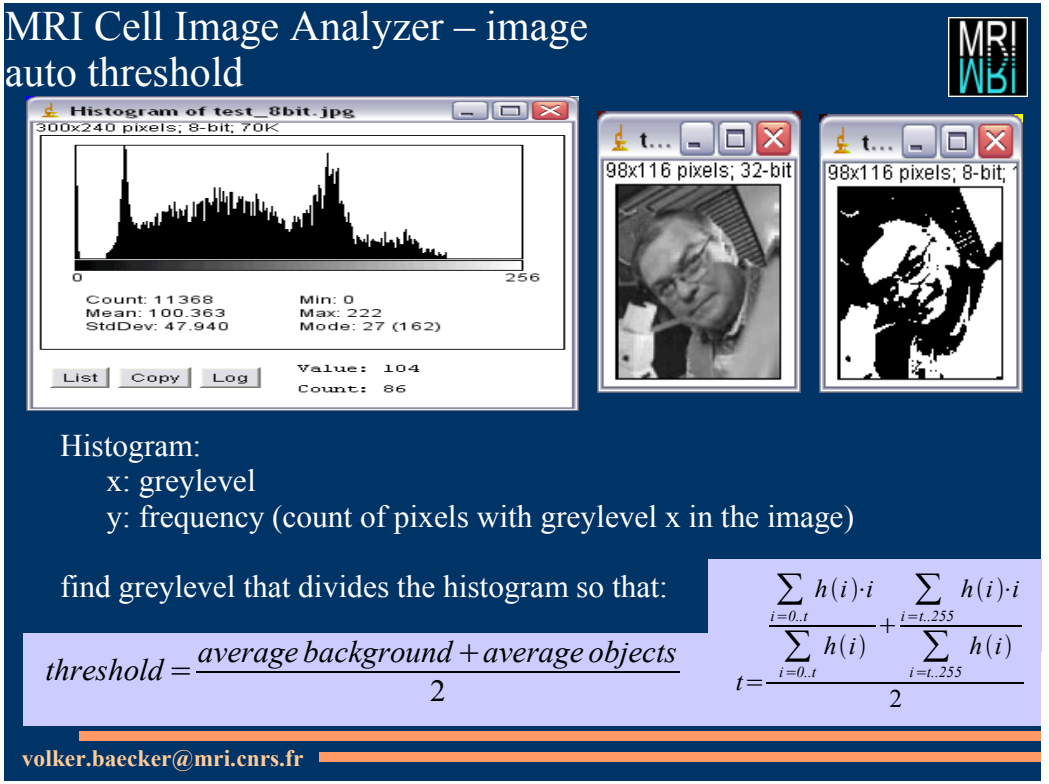
The image is then convolved with the kernel.

This means each pixel value the replaced by the weigthed sum of his value and the values of the pixels in its neighborhood.

The weights are given by the values in the normalized kernel.



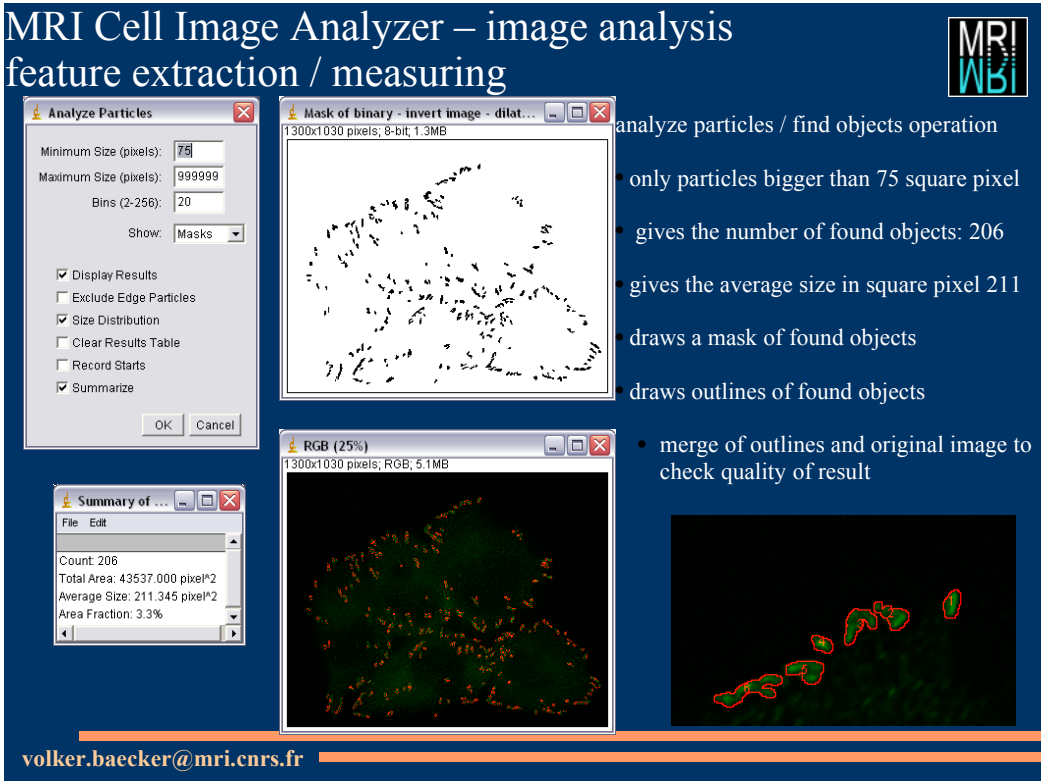
The auto-threshold creates a mask. Since afterwards objects are expected to be black and background to be white, the image is inverted. Finally a dilate operation is applied since the threshold has diminished the sizes of the objects a little bit.



How does the autothreshold work?

The histogram of an image shows the greylevels on the x-axis and the count of each greylevel in the image on the y-axis.

The autothreshold uses the grey level that divides the histogram so that two times the grey level is equal to the sum of the average background and the average object intensities.



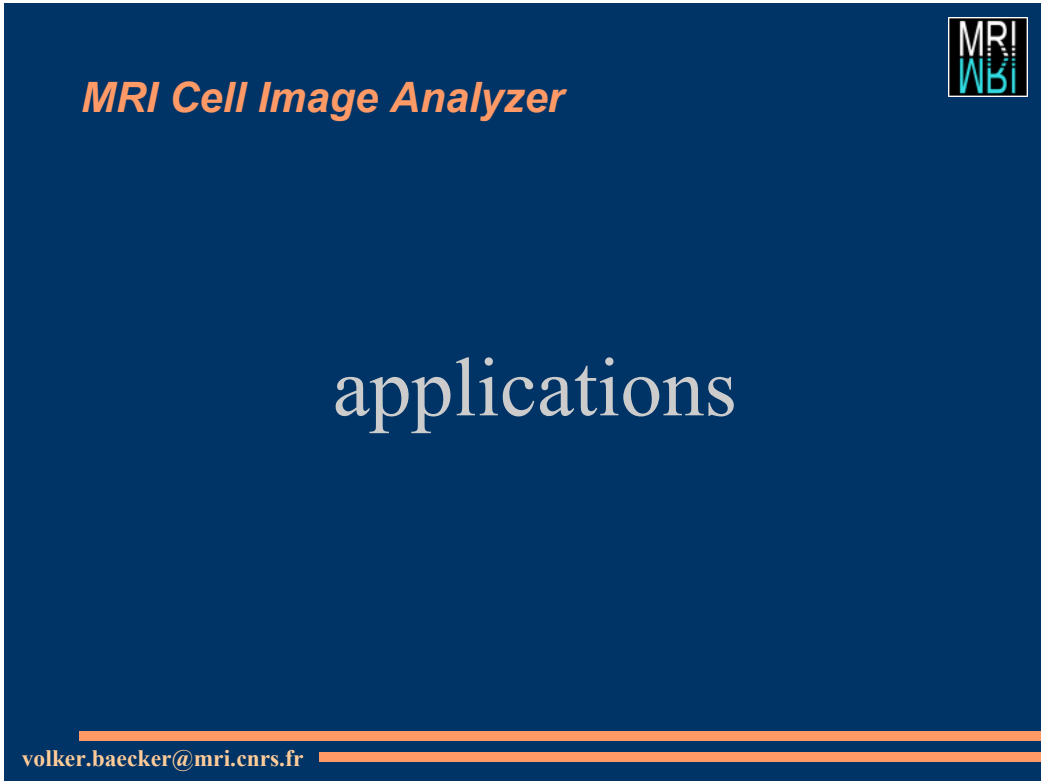
The find objects operation will extract the features we asked for. It will measure the size of all objects and calculate the average.

We restrict the objects to be taken into account by their size.

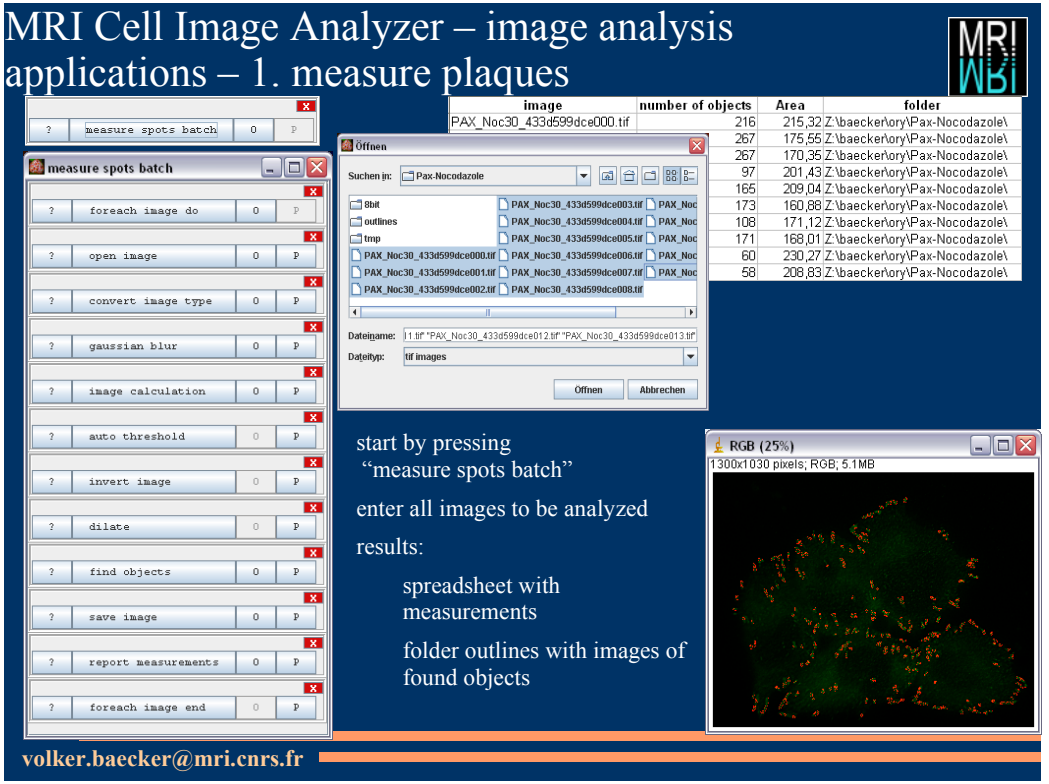
There are 206 plaques in the image. Their average size is 211 square pixel.

The find objects operation creates a mask of the objects found and an outlines image of them.

By merging the outlines image with the input image we can control if the detection of the plaques worked as expected.



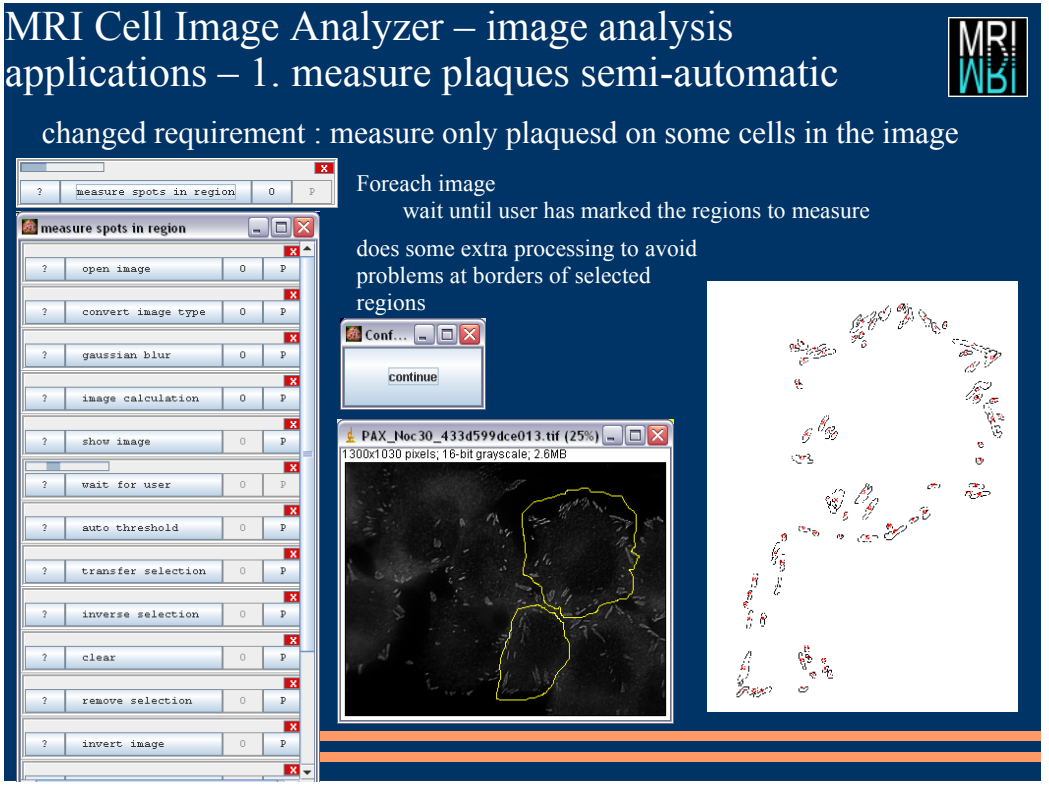
Some of the applications implemented will be presented now.



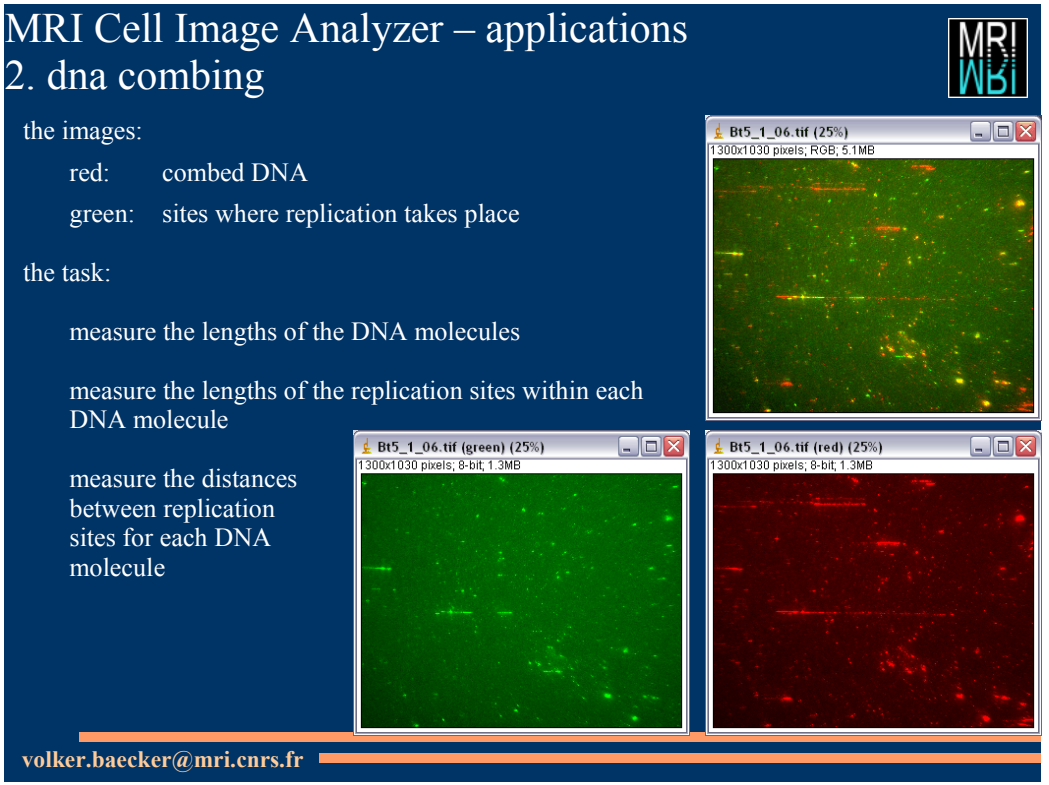
One application created is the automation of the solution to the count and measure plaques problem.

When you start the application you can enter a folder or a number of images.
You will be asked where to save the result spreadsheet.
All images will be processed without further interactivity.

The result is the spreadsheet with the measurements and a folder named outline into which the outlines images of the found plaques are saved.

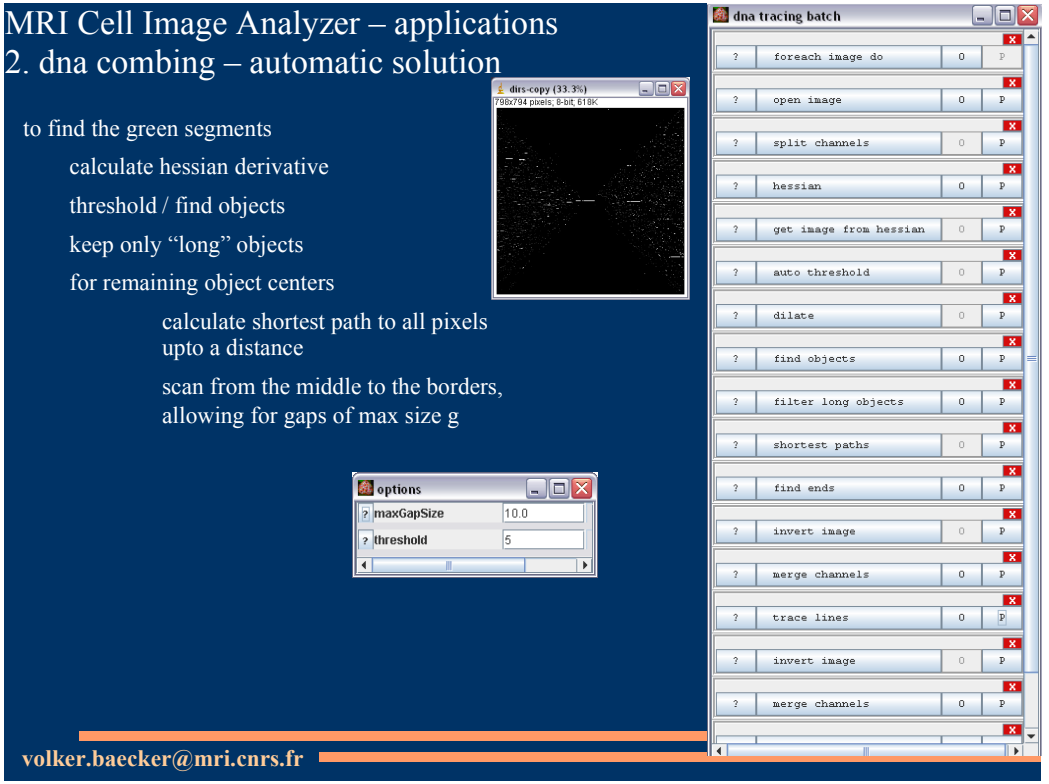


A modified version lets the user select regions before proceeding.



The images contain unfolded DNA molecules stained in red. Sites in the molecules where replication takes place are stained in green.

The task is to measure the lengths of the DNA molecules, the lengths of the replication sites and the distances between neighboring replication sites in a molecule.



I first created an automatic solution It starts by finding the green segments.

To do this it calculates the second order derivate (hessian) to find edged in the image.

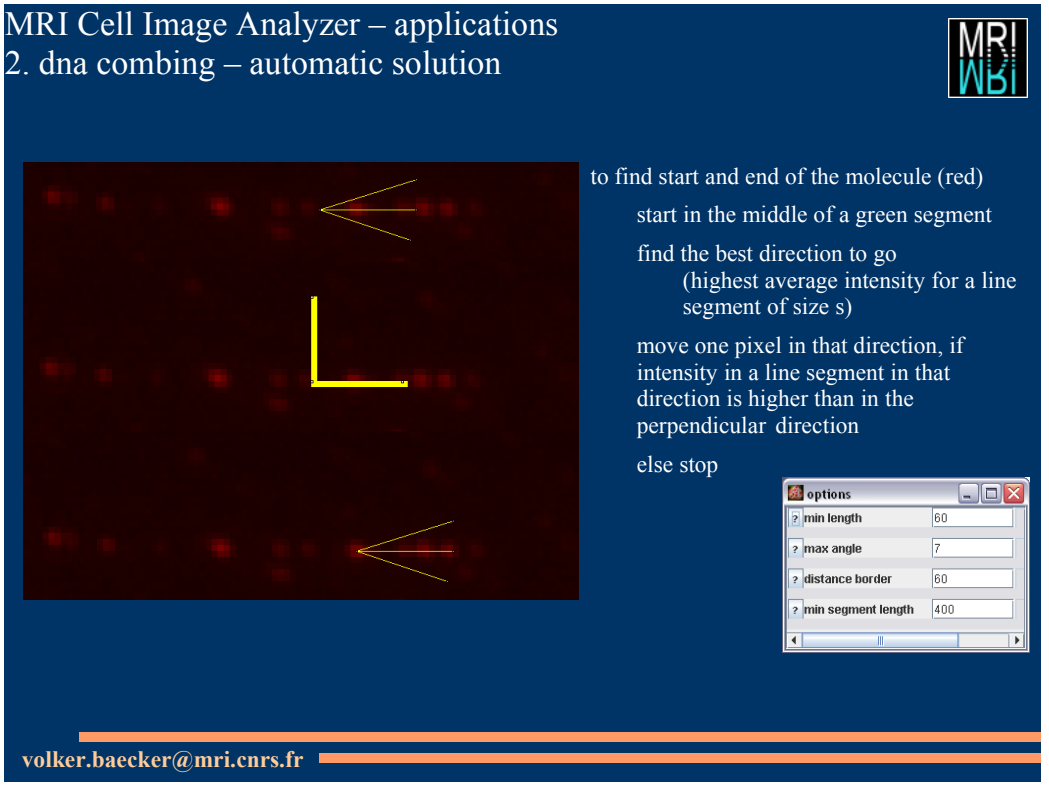
A threshold and the find objects operation are applied.

Only long objects are kept. To judge if an object is long the width and length of the object are compared.

For the remain object centers calculate the shortest paths to all pixels upto a distance.

With help of the shortest path image trace the segment from the middle to the borders allowing for gaps of a give maximum size.

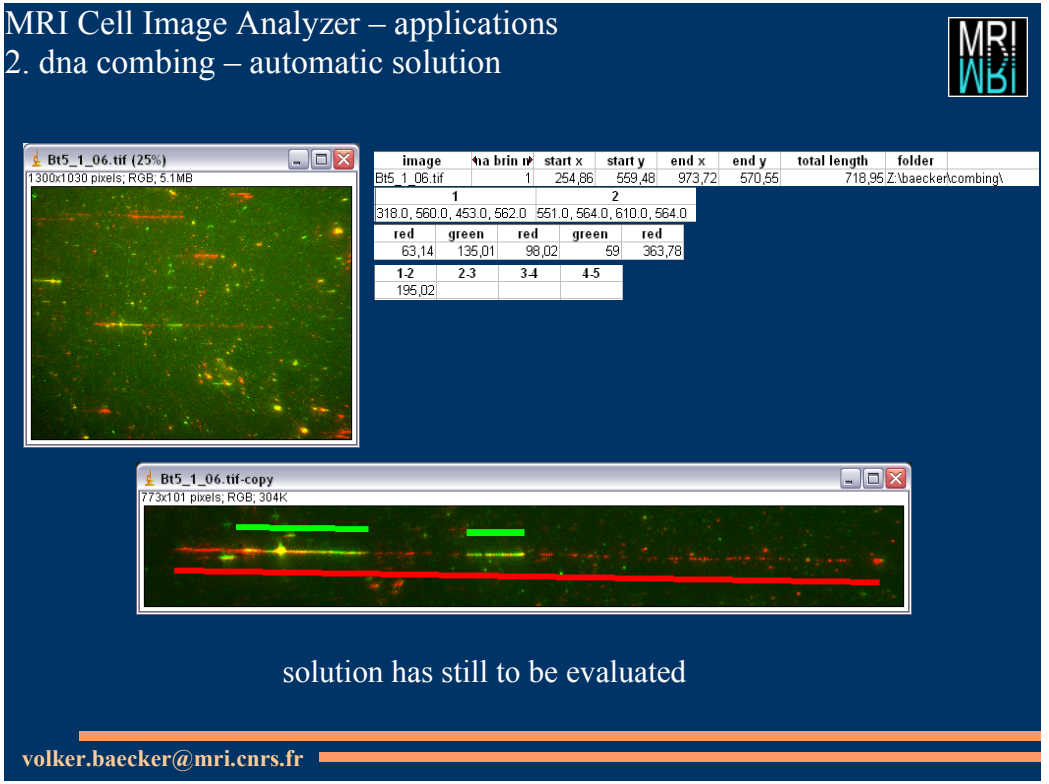
The use of the hessian derivate and the calculation of the shortest paths have been copied from Erik Meierings NeuronJ. The DNA combing task is similar to the neurite tracing task, however there are more and larger gaps in case of the DNA combing task.



One difficulty is that the molecules are not perfectly straight. So searching for straight lines doesn't work. The other difficulty is that the red segments can have very large gaps and that much (biochemical) noise is present in the image.

Since the green segments lie on the red segments I start from the middle of a green segment. A short line segment is put in this point it is turned around the point between a min and max angle. The direction with the highest average intensity is selected and the tracing continues there, if the average intensity is still higher in that direction than in the perpendicular direction. Otherwise the end has been found.

The molecules ends must have a certain distance from the images' borders and they must have a minimal length.

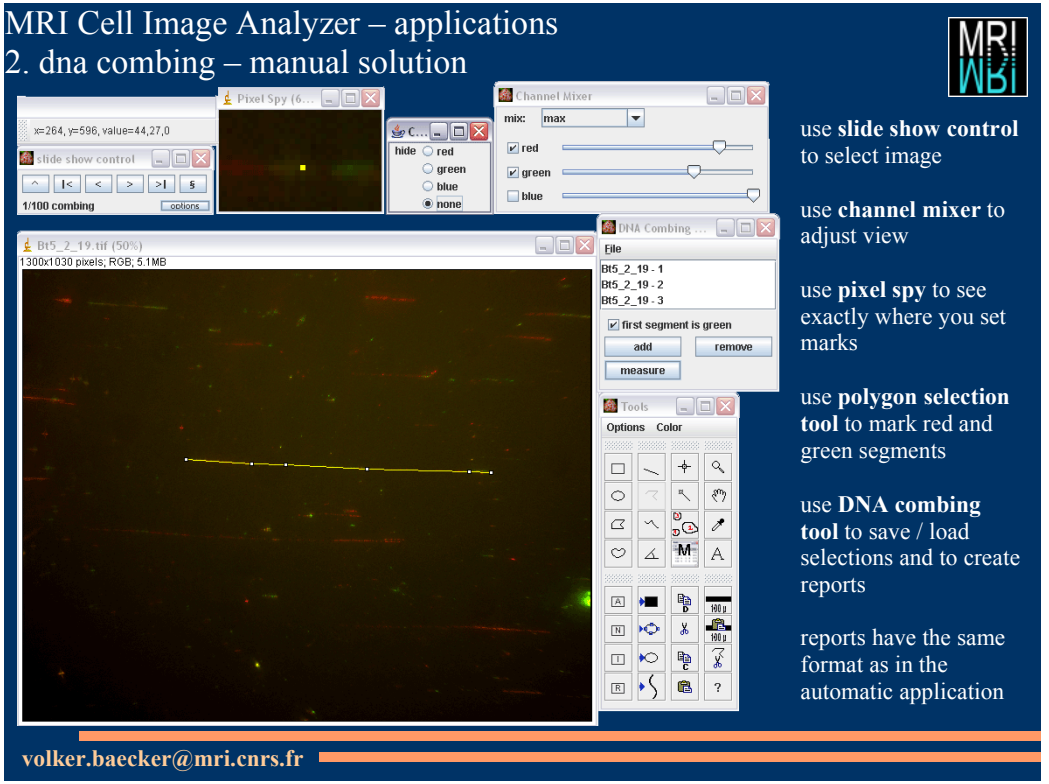


The results are written to a spreadsheet.

In the example the segments have been found.

The solution has still to be evaluated.

To do so we need images for which the segments have already been measured.



To create this data the measurements can be made manually, using the interactive tools.

The slide show control to open one image after the other.

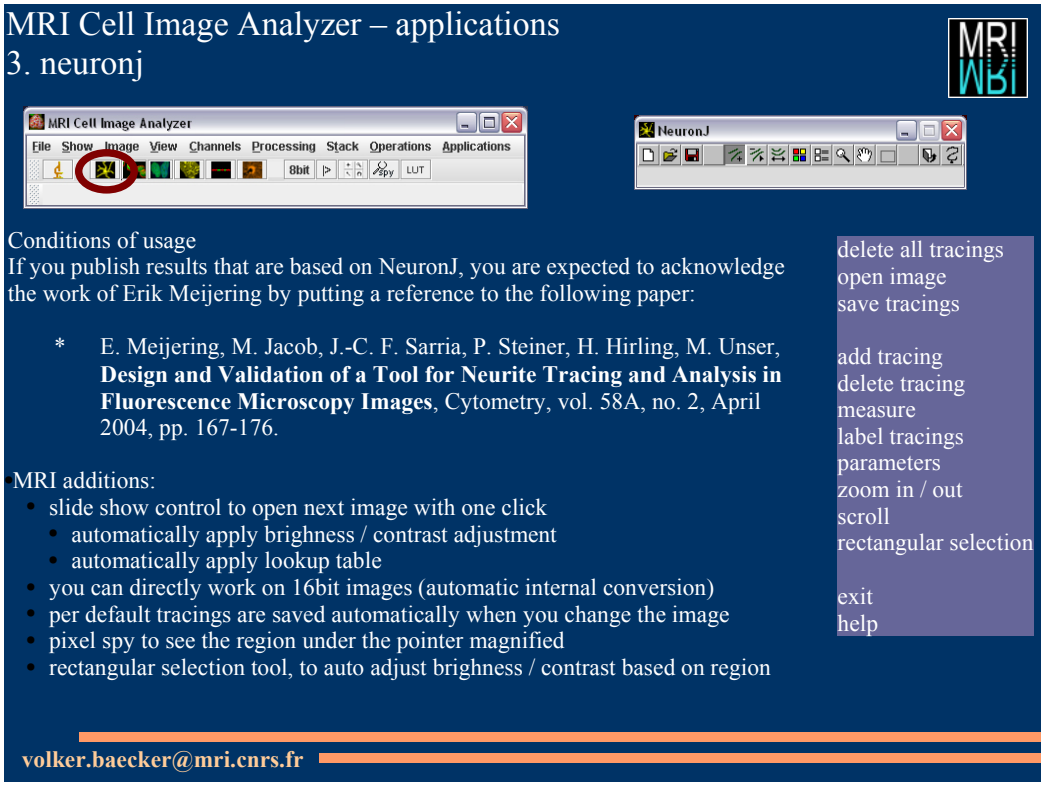
The channel mixer to adjust the display and to switch of the green or red channel.

The pixel spy to see exactly where marks are set.

The polygon selection to mark the green and red segments.

The DNA combing tool to save and load selections and to create reports.

The reports created have the same format as in the automatic application.



Open the NeuronJ toolbar by clicking on the neuron icon.

If you publish results that have been obtained using NeuronJ you have to cite the publication of Erik Meijering.

The MRI version has the following additions:

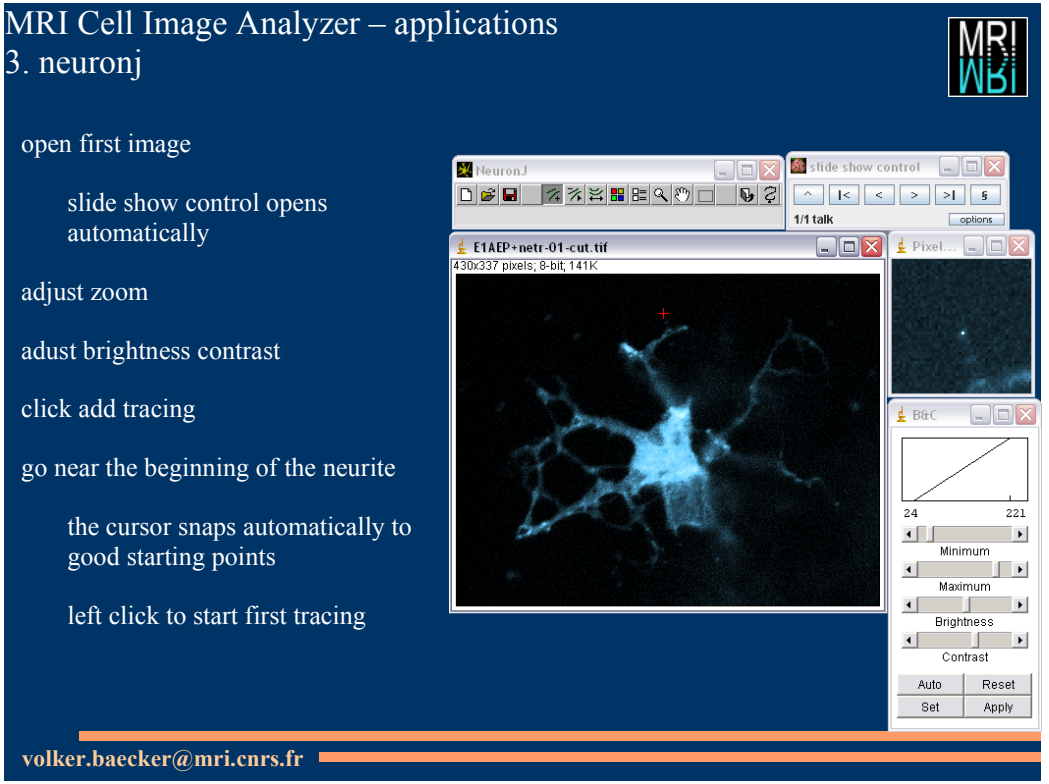
It is shown in its own window not as a toolbar in the imagj window.

You can use the slide show control to open images and automatically apply brighness / contrast adjustment and a look up table.

You can directly work on 16bit images, they are converted internally.

Tracings are per default saved automatically when you change the image.

You can use the rectangular selection tool to auto adjust brightness / contrast

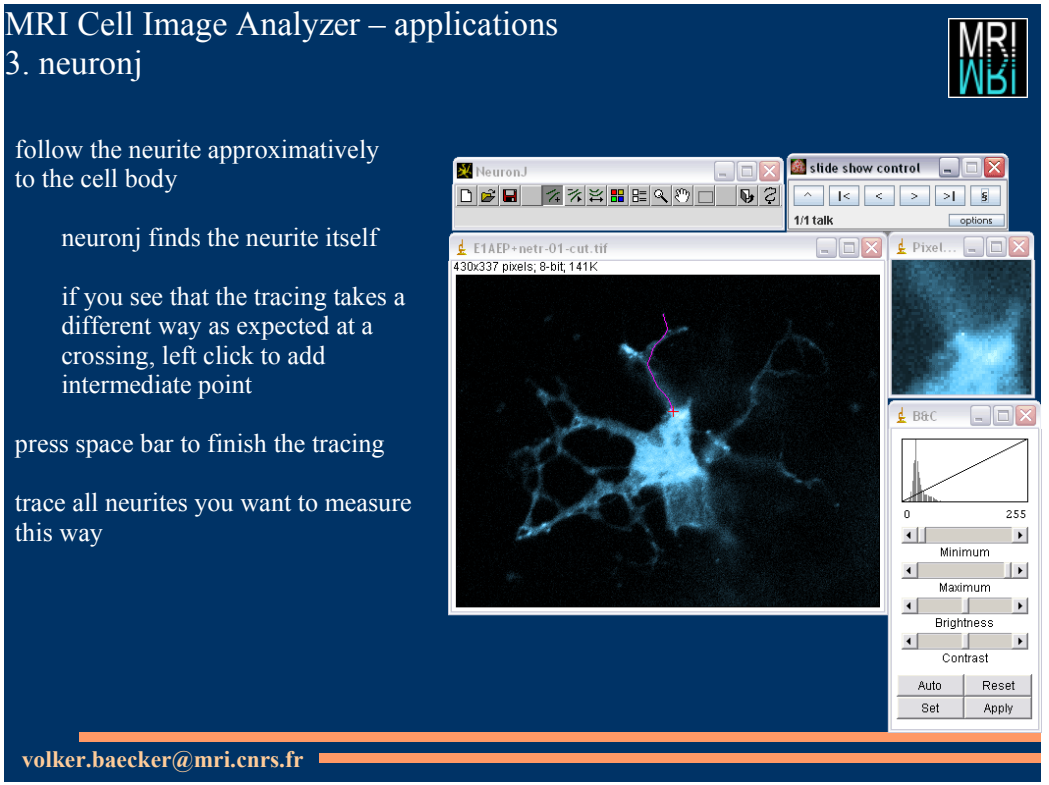


Open the first image from the neuronj window. The slide show control will automatically be opened.

Adjust the zoom and adjust brightness and contrast.

Click the add tracing button. Go near the end of a neurite. The cursor snaps automatically to a good starting point.

Left-click to start the tracing.

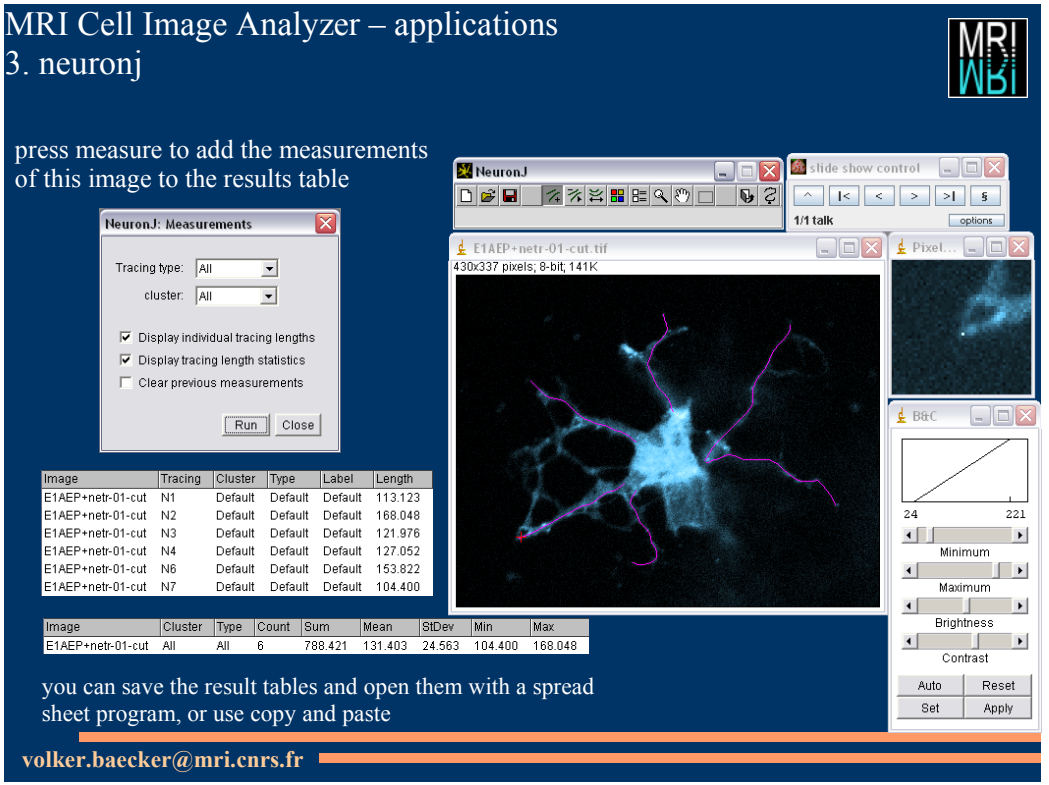


Follow the neurite approximatively to the cell body. Neuronj finds the exact neurite itself.

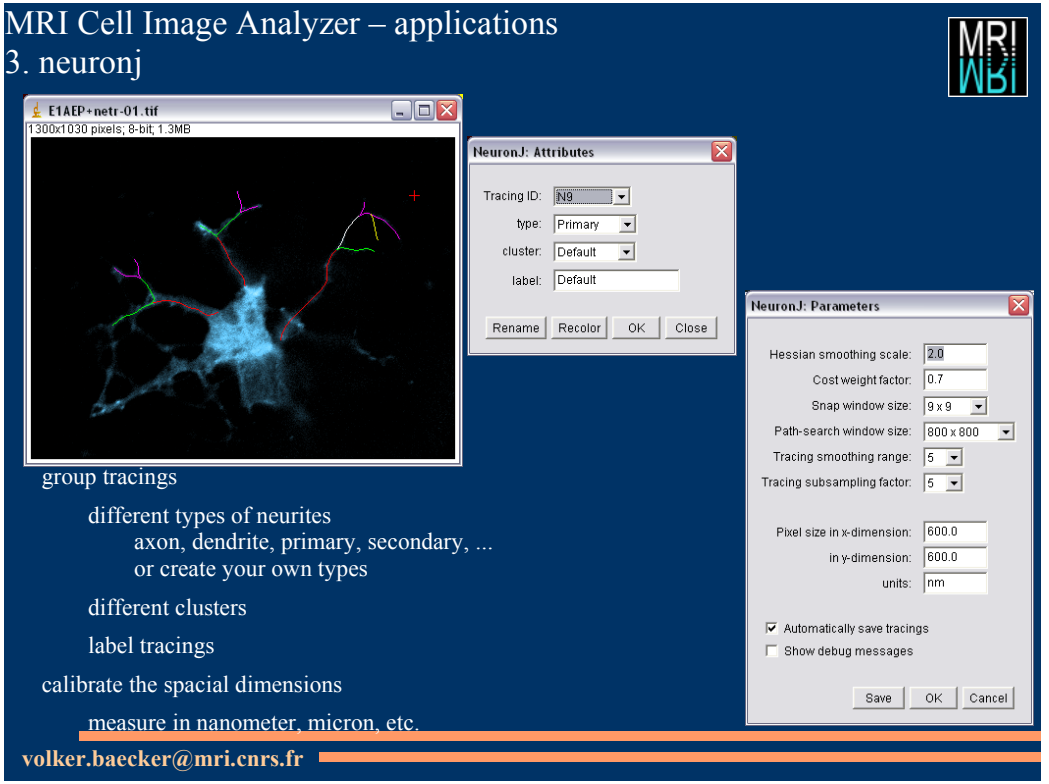
If you see that the tracing takes a different way as expected at a crossing, left click to add an intermediate point.

Press space bar to finish the tracing.

Trace all neurites you want to measure this way.



Open the measure dialog. You can keep the window open. Click run to add the measurements for this image to the results table.



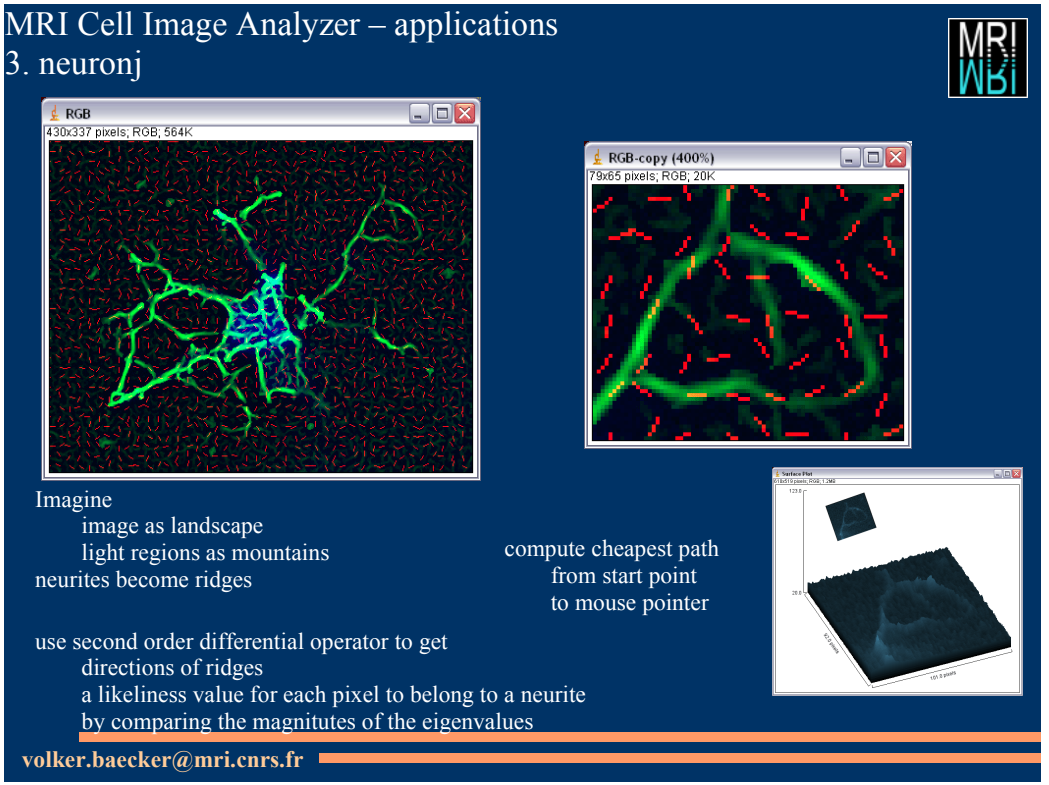
You can group tracings by their type, like axon, dendrite, primary, secondary, etc.

Different types of tracings are displayed in different colors.

You can group them into different clusters

You can label them with custom names

In the NeuronJ parameter dialog you can calibrate the spacial dimensions to measure in nanometer or micron instead of measuring lengths in pixels.



How does it work?

Imagine the image as a landscape where light regions are mountains. In the landscape neurites are ridges.

In a preprocessing step a second order differential operator (hessian) is used to get the directions of the ridges and to calculate a likeliness for each pixel to belong to a neurite.

When a tracing is started, the cheapest path from the start point to the mouse pointer is calculated using the data from the preprocessing step.

The red marks in the image reflect the directions of the ridges.

MRI Cell Image Analyzer – applications
4. comparing intensities

what is the proportion of fluorescence between nuclei and cytoplasm in the first image?

the second image is used to identify the nuclei

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what is the proportion of fluorescence between nuclei and cytoplasm in the first image? The second image is used to identify the nuclei.

MRI Cell Image Analyzer – applications
4. comparing intensities

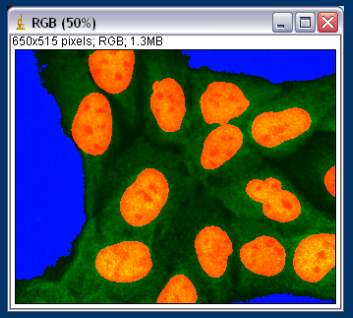
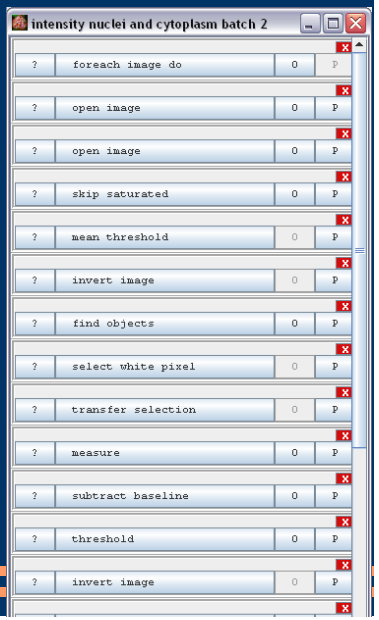


image	icn factor	percent nuclei	percent cytoplasm	av. nuclei intensity	av. cytoplasm intensity
dapi 2.tif	0,85	0,46	0,54	112,67	61,99

- threshold nuclei image
- select objects
- transfer selection to input image
- measure selection
- subtract adaptive baseline to zero background
- select none zero pixels of the image in the original image
- measure
- subtract nuclei intensity
- calculate proportions



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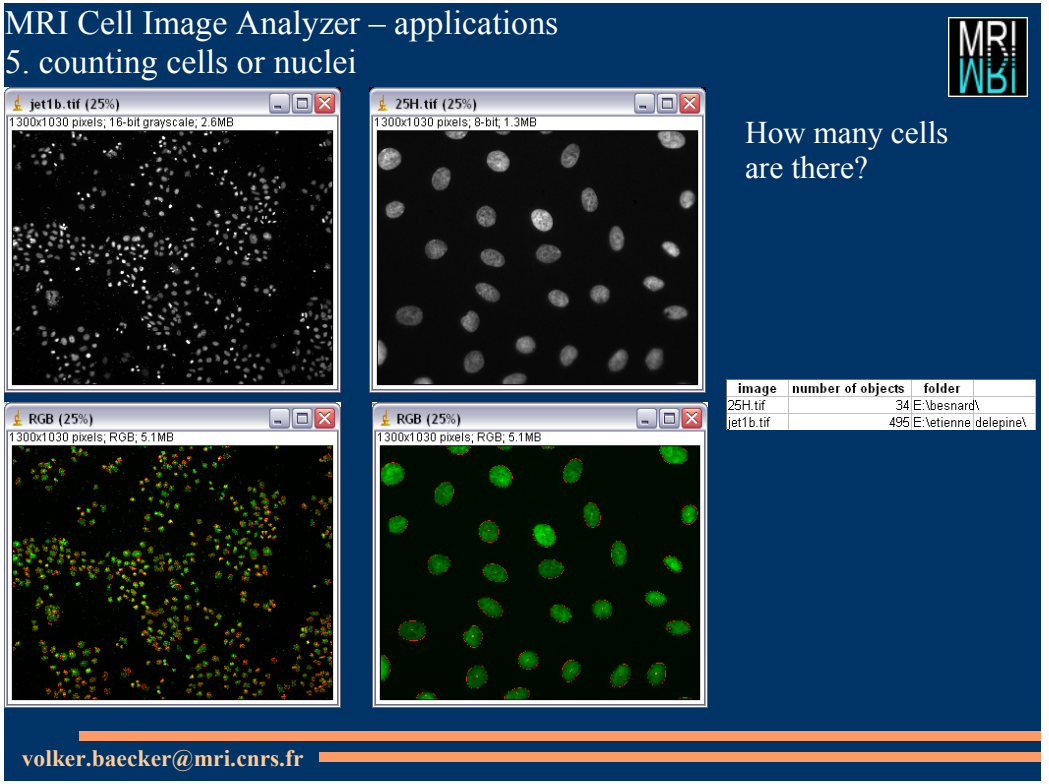


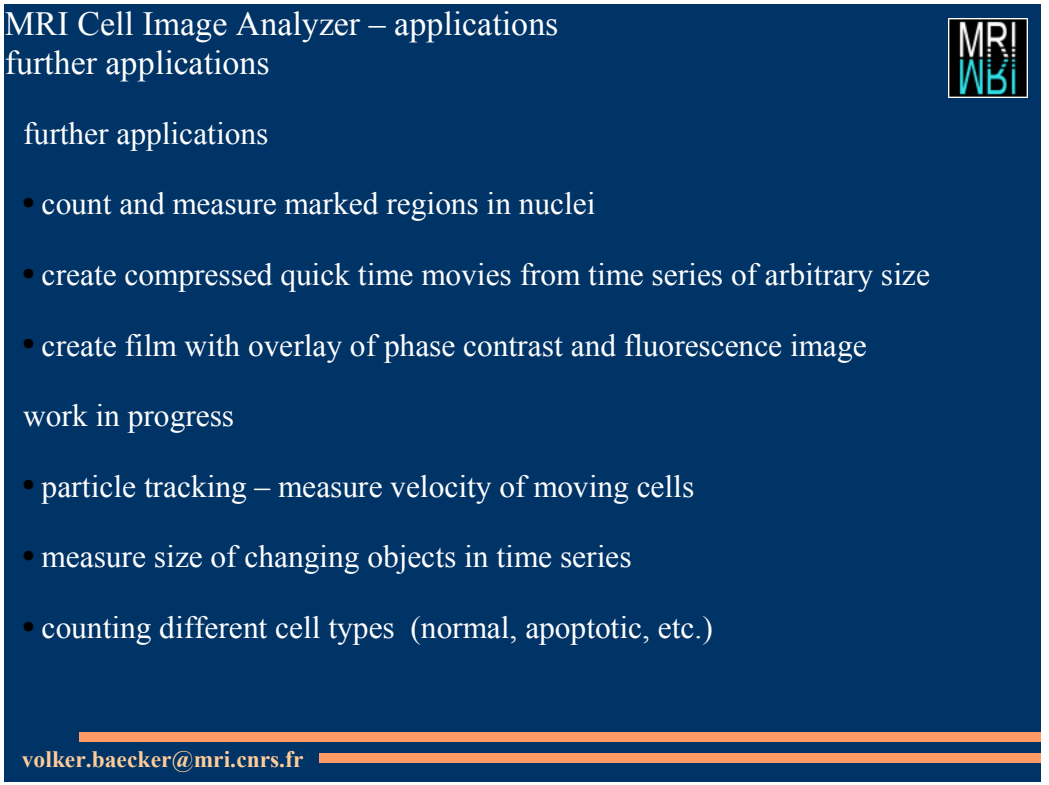
Another application counts objects in the image.

How many are there in the two images?

In the first one there are 495 and in the second one there are 34.

It works perfectly when objects are convex and well seperated. It works as well when objects touch or overlap.

A watershed operation is used to seperate the objects. This works good although it doesn't always yield the right results.



Further implemented applications:

Count and measure stained regions per nucleus.

Create compressed quick time moveis from time series of arbitrary length. This hasn't been possible with standard software, because all images are loaded into RAM to create a film which is not possible for long series.

Createa film with an overlay of the a phase contrast and a fluorescence image.

Work in progress:

Particle tracking in time series. Measure the velocites and path lengths of moving particles.

Measure how the size of an object changes in a time series.

The cell counter tool will allow you to select some cells of one kind and will then search for similar objects in the image.

MRI Cell Image Analyzer

summary and outlook

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MRI Cell Image Analyzer – conclusion and outlook

- MRI Cell Image Analyzer
  - is an adequate tool for the rapid devolpment of image analysis applications
  - finding a solution and creating the application go hand in hand with it
  - it can be used by biologists and application developpers together
  - batch applications can treat all images to be analyzed in one run
  - since it is based on imagej a lot of functionallity is immediately available, including plugins for specific tasks.
- Sometimes automatic solutions are not (yet) adequate
  - the possibility for the user to make corrections has to be build in
  - or at least appropriate tools for the manual treatment can be provided
- A number of image analysis applications has been realized.

  Showing the value of the bottom up approach
    - where specific problems are solved and
    - solutions are assembeled to a framework

So let us try to solve your image analysis problem now...

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MRI Cell Image Analyzer – conclusion and outlook

- what comes next depends on you...
- there seems to be a need for a virtual stack processing
  - to treat very big sequences (stack, time series)
  - virtually like any other images without the need to load them entirely
  - only showing data necessary at one moment
  - working on the disk behind the scenes
  - the concept is known from text, sound and movie editing and is called a streaming editor there

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MRI – announcement

- for doing deconvolution at MRI
  - you will soon be able to upload your images to our fileserver and to download results
  - this will be possible from all MRI analysis pcs
  - if you want to do it from other pcs, for example in your lab
    - please tell us
      - we need to know the ip address
      - we need to install and configure a client software

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MRI Cell Image Analyzer – the last slide

Thank you for your attention!

? Questions ?

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MRI Cell Image Analyzer – literature and links

ImageJ

Abramoff, M.D., Magelhaes, P.J., Ram, S.J. "Image Processing with ImageJ". Biophotonics International, volume 11, issue 7, pp. 36-42, 2004.

Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://rsb.info.nih.gov/ij/ , 1997-2005.

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Visual programming

Visual Programming Research, Introduction and Philosophy, Dr. R. Mark Meyer, Computer Science Department, Canisius College, July 1, 1999, http://www-cs.canisius.edu/~meyer/VP/intro.html

Image Processing

Look Up Tables - HyperMedia Image Processing Reference, The University of Edingburgh, Look Up Tables and Colourmaps, http://www.cee.hw.ac.uk/hipr/html/colmap.html

Histogram - HyperMedia Image Processing Reference, The University of Edingburgh, Intensity Histogram, http://www.cee.hw.ac.uk/hipr/html/histgram.html

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MRI Cell Image Analyzer – literature and links

Image Processing

Contrast Stretching - HyperMedia Image Processing Reference, The University of Edingburgh, Contrast Stretching,,
http://www.cee.hw.ac.uk/hipr/html/stretch.html

Thresholding - HyperMedia Image Processing Reference, The University of Edingburgh, Thresholding,,
http://www.cee.hw.ac.uk/hipr/html/threshld.html

Image Arithmetic - HyperMedia Image Processing Reference, The University of Edingburgh, Image Arithmetic,,
http://www.cee.hw.ac.uk/hipr/html/arthops.html

color models (RGB, CMYK) – Wikipedia, Color space, http://en.wikipedia.org/wiki/Color_space

Gaussian Blur – Algorithms, Ian Craw and John Pulham, University of Aberdeen,
http://www.maths.abdn.ac.uk/~igc/tch/mx4002/notes/node99.html

Dilation - HyperMedia Image Processing Reference, The University of Edingburgh, Dilation,,
http://www.cee.hw.ac.uk/hipr/html/dilate.html

Autothreshold – wikipedia, Thresholding (image processing),
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MRI Cell Image Analyzer – literature and links

Hessian - IEEE TRANSACTIONS ON IMAGE PROCESSING, VOL. 7, NO. 3, MARCH 1998 353,
Adaptive Smoothing Respecting Feature Directions, Ren´e A. Carmona, *Member, IEEE,* and Sifen Zhong
(http://www.mi.uib.no/~mariusl/carmona.pdf)

Pattern Recognition

H. Niemann: Klassifikation von Mustern. Springer Verlag, Berlin, 1983.
(http://www5.informatik.uni-erlangen.de/Personen/niemann/klassifikation-von-mustern/m00links.html)

R.O. Duda, P.E. Hart: Pattern Classification and Scene Analysis. John Wiley & Sons, Inc., 1973.

NeuronJ

E. Meijering, M. Jacob, J.-C. F. Sarria, P. Steiner, H. Hirling, M. Unser, Design and Validation of a Tool for Neurite Tracing and Analysis in Fluorescence Microscopy Images, Cytometry, vol. 58A, no. 2, April 2004, pp. 167-176.
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