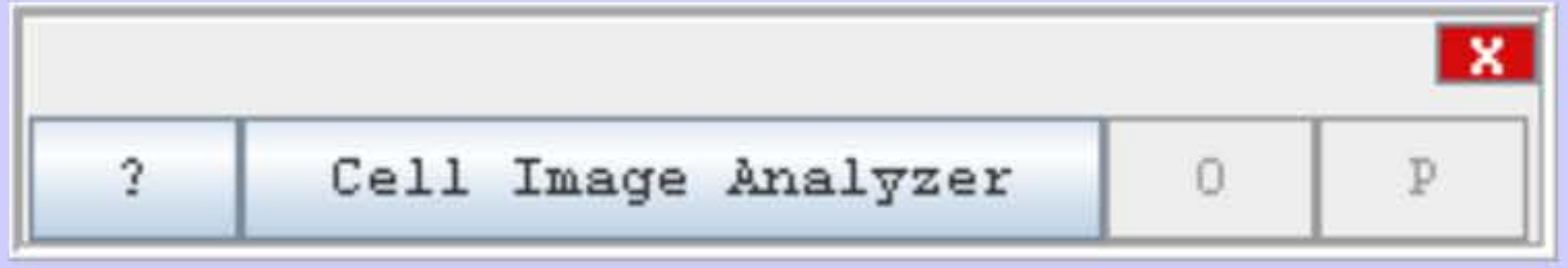


A visual scripting interface for ImageJ and its usage at the microscopy facility Montpellier RIO Imaging

Volker Baecker and Pierre Travo

19.05.2006

Montpellier RIO Imaging

Overview

A visual scripting interface for ImageJ

- introduction and motivation
- using visual scripting
  - simple applications
  - batch applications
  - interactive applications
- extending the framework
  - adding operations
  - accessing ImageJ methods
- the implementation

... and its usage at the microscopy facility MRI

- DNA combing application
- measuring intensity ratios
- measuring fluorescent stained proteins
- counting cells and nuclei
- automation of simple tasks
- further project
- conclusions

Who am I

- Born: 29.08.1970
- Nationality: German (living in France since Nov. 2003)

- 2000
  Master thesis in computer science at the University of Dortmund:
  “A development environment for cellular automata with fuzzy rules”

- 2000 – 2003
  Software developer and consultant for object oriented systems
  George Heeg eK

- Nov. 2003 – Feb. 2004
  Centre de Recherches de Biochimie Macromoléculaire,
  Structural Bioinformatics and Molecular Modelling, Andrey Kajava

- From Feb. 2004
  Software development at the Microscopy Facility: Montpellier RIO Imaging

Montpellier RIO Imaging

Responsable

PierreTravo, IRCE CNRS

- regional imaging facility
- 4 sites
- 23 microscopes
- 420 users
- 25413 hours in 2005
- 3M images in 2005

- promote the usage of microscopy
- participate in the development of microscopy
- provide training

MRI-EDU

Composition variable
Toutes opérations de formation et communication
Resp. J. Cau

MRI-DEV

Corine Tran-Aupiais
IE2 CNRS
Optronique

Optique adaptative

Olivier Miquel
T3 CNRS
Systèmes Réseaux

Soutien à la plate-forme

Sylvain de Rossi
IE2 INSERM
Optique et systèmes LASER

Soutien à la plate-forme

Volker Bäcker,
IE CDD
Conception développement

MRI Cell Image Analyzer

Montpellier RIO Imaging

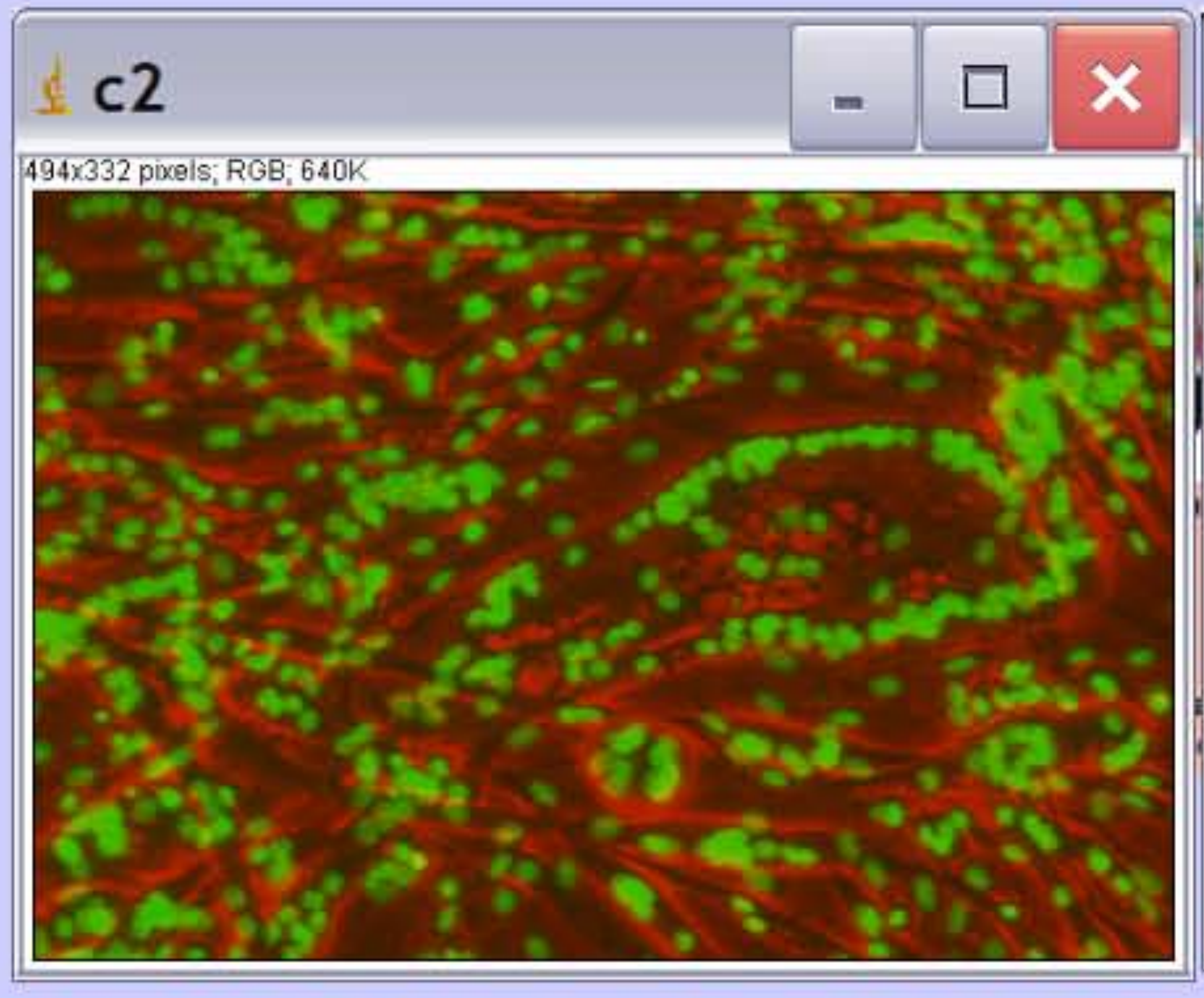
Responsable

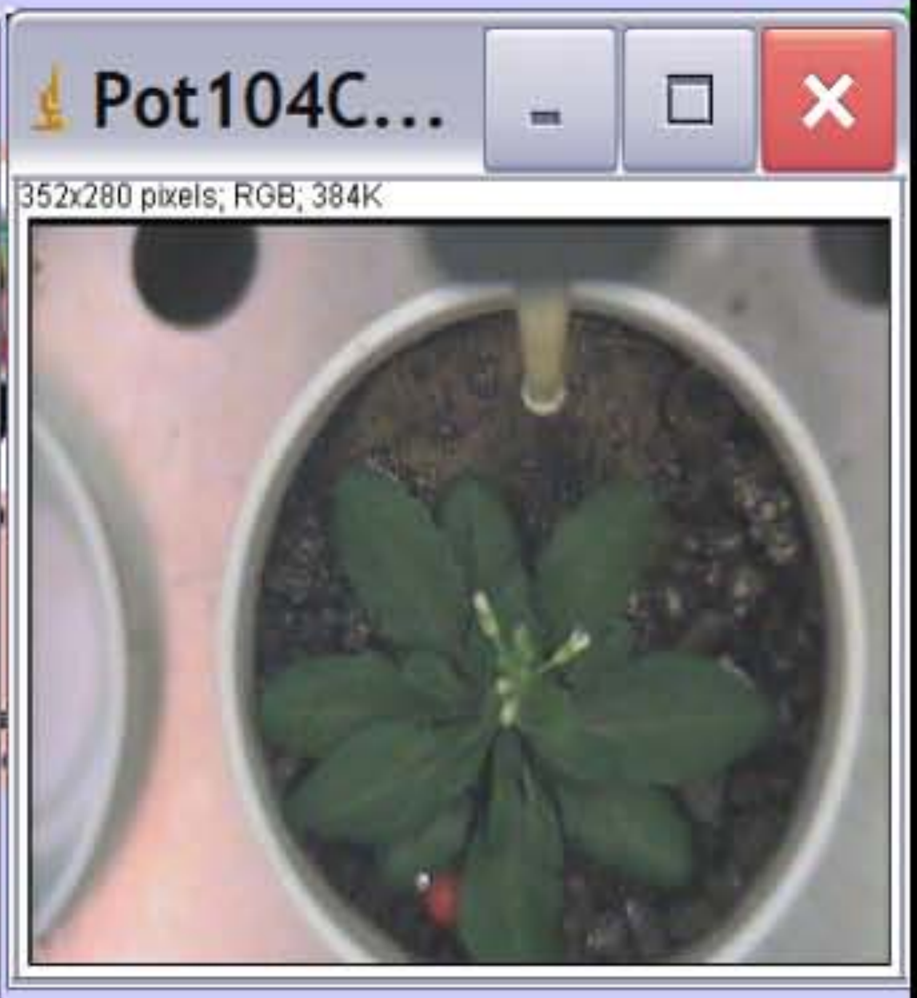
PierreTravo, IRCE CNRS

- regional imaging facility
- 4 sites
- 23 microscopes
- 420 users
- 25413 hours in 2005
- 3M images in 2005

wide range of mostly biological applications
images from sub-cellular to entire organisms

- promote the usage of microscopy
- participate in the development of microscopy
- provide training





The problem

robotized acquisition,
time series, volume images

- large amounts of data

manual analysis

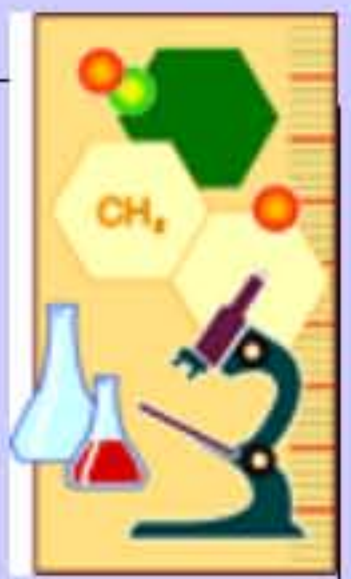
- time consuming
- biased results?

analysis is the bottleneck

⇒ automatic analysis needed

wide range of

- analysis needs
- image qualities



⇒ analysis must use a priori knowledge

if automatic analysis not possible

- partial automation can augment efficiency
- let the user only do what the software can't do

⇒ semi-automatic analysis needed

Problem / Requirements

wanted:

rapid prototyping framework for image analysis applications

requirements:

- interactive experimentation to find solutions
- create interactive and batch applications
- build prototype-applications from existing operations rapidly
- extendable - add new operations
- allow to parametrize and run applications
- easy to use for end user

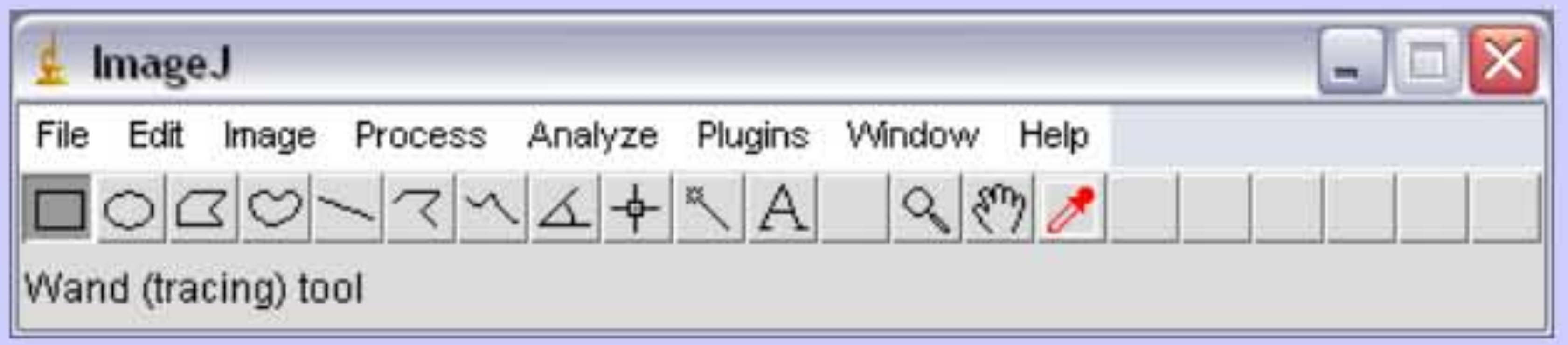
Process

process:

- use framework
  - to create prototype solutions on demand
  - in close collaboration with biologist
  - extend framework,
    when necessary for a project
    - **only then**

- eventually create full featured application

ImageJ



Is ImageJ the desired framework?

- interactive experimentation
  - solid image processing / analysis framework
- create interactive and batch applications
- allow to parametrize and run applications
  - macros
- extendable, configurable
  - plug-ins, source code available, public domain

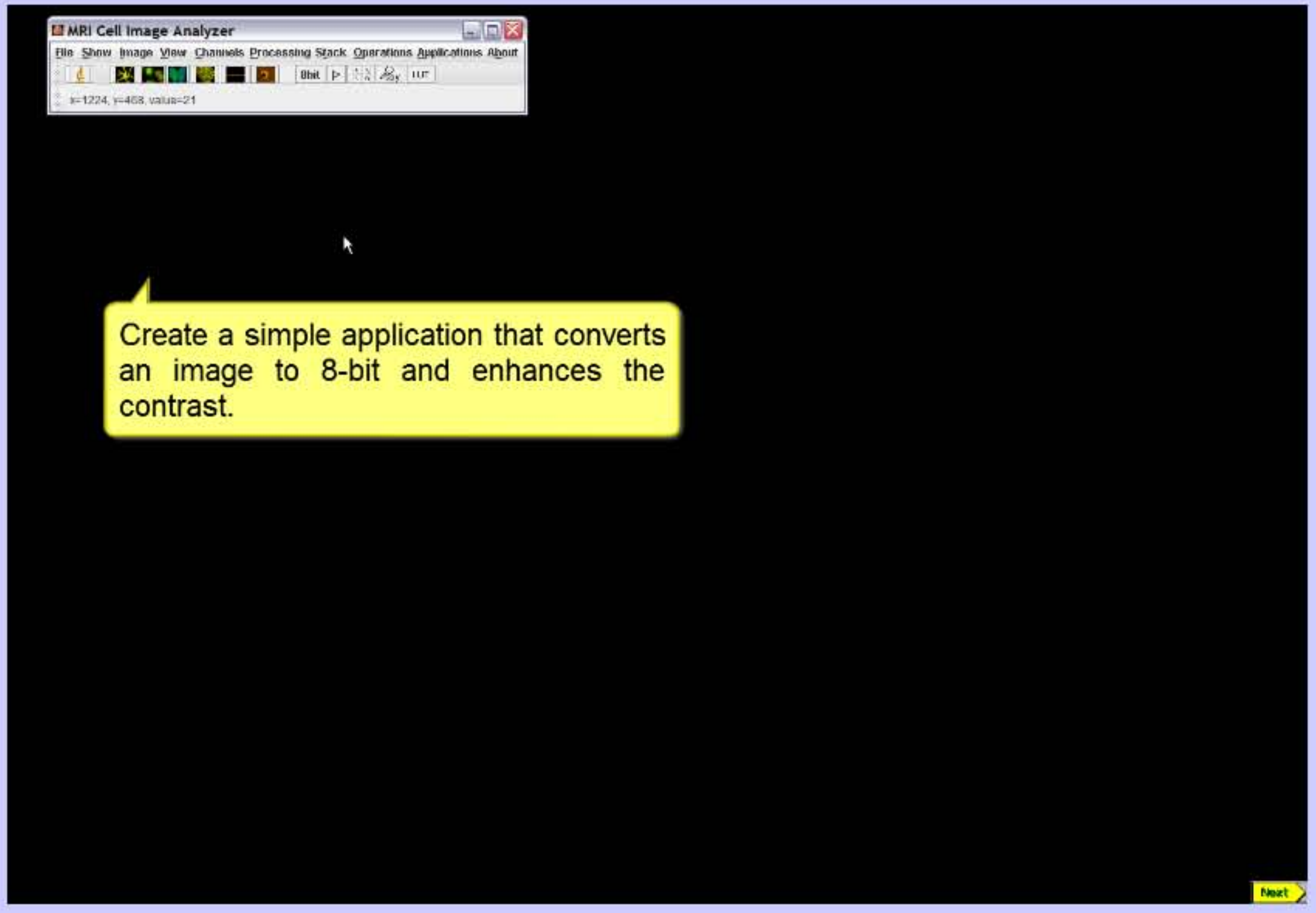
- build prototype-applications from existing operations rapidly
  - yes, but macro language not understandable for end user
- easy to use
  - yes, but users have to learn command shortcuts or search in menus

→ great for image analysis specialist

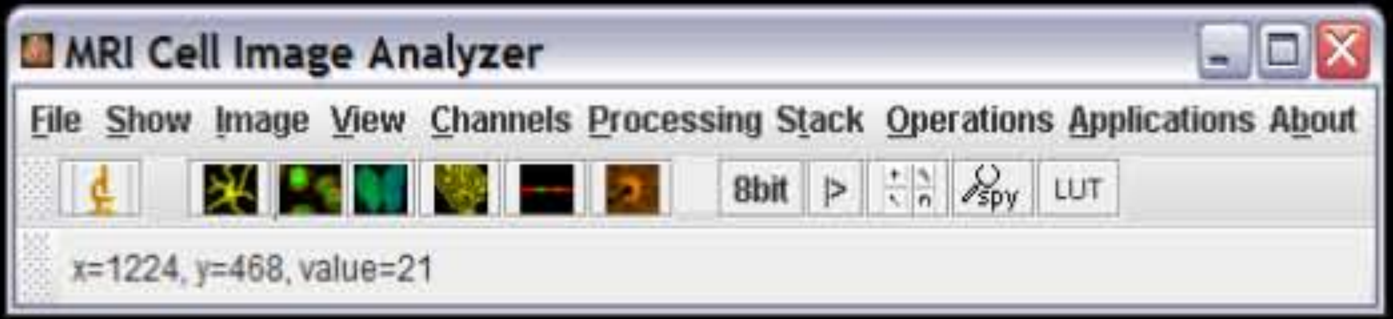
→ some aspects too technical for end user

Using visual scripting

- create a simple application


MRI Cell Image Analyzer
File Show Image View Channels Processing Stack Operations Applications About
8bit
LUT
x=1224, y=468, value=21
Next

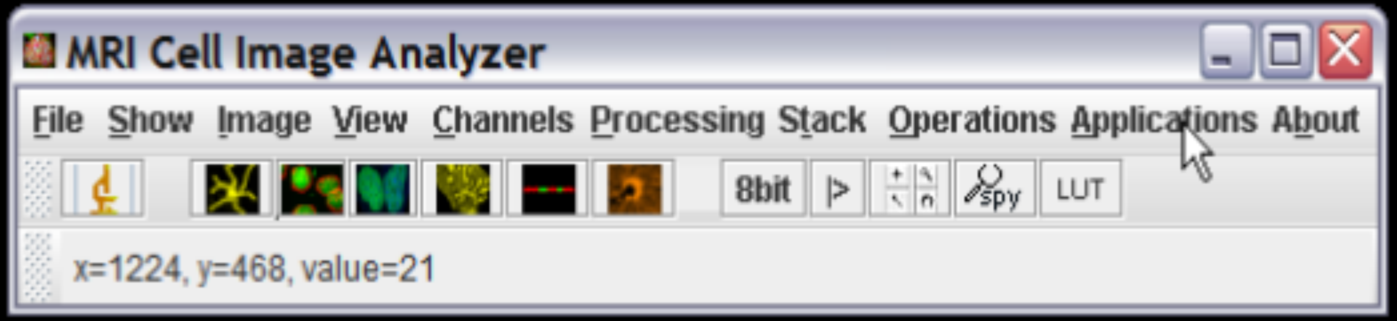
Create a simple application that converts an image to 8-bit and enhances the contrast.


MRI Cell Image Analyzer
File Show Image View Channels Processing Stack Operations Applications About
8bit
LUT

x=1224, y=468, value=21

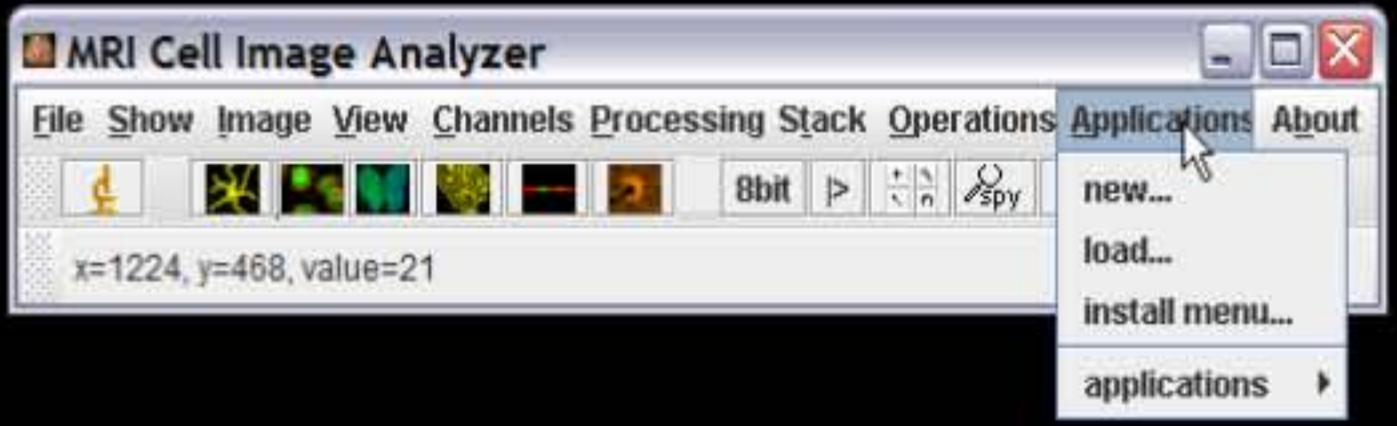
Create a simple application that converts an image to 8-bit and enhances the contrast.

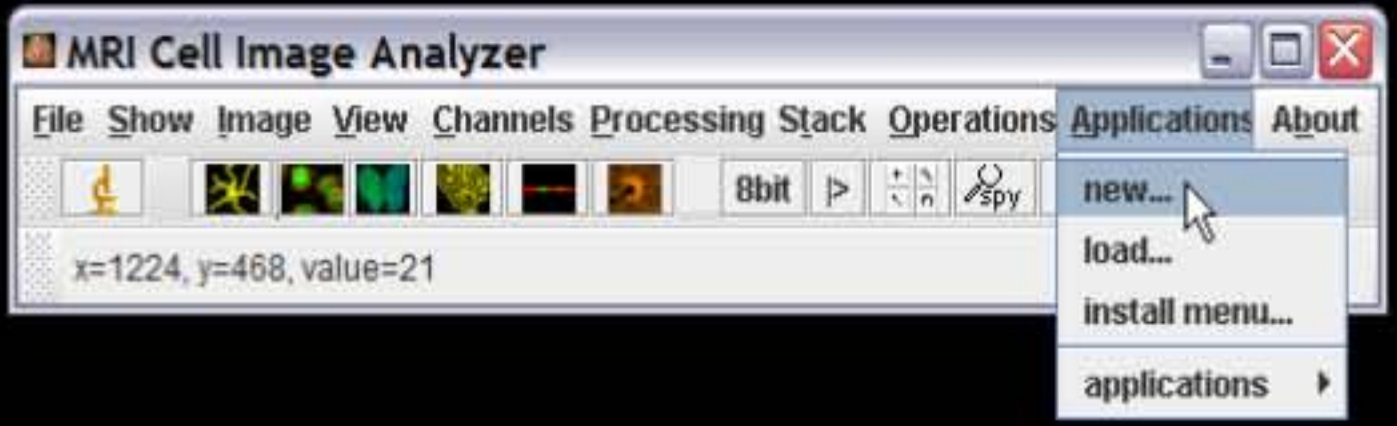
Next



x=1224, y=468, value=21

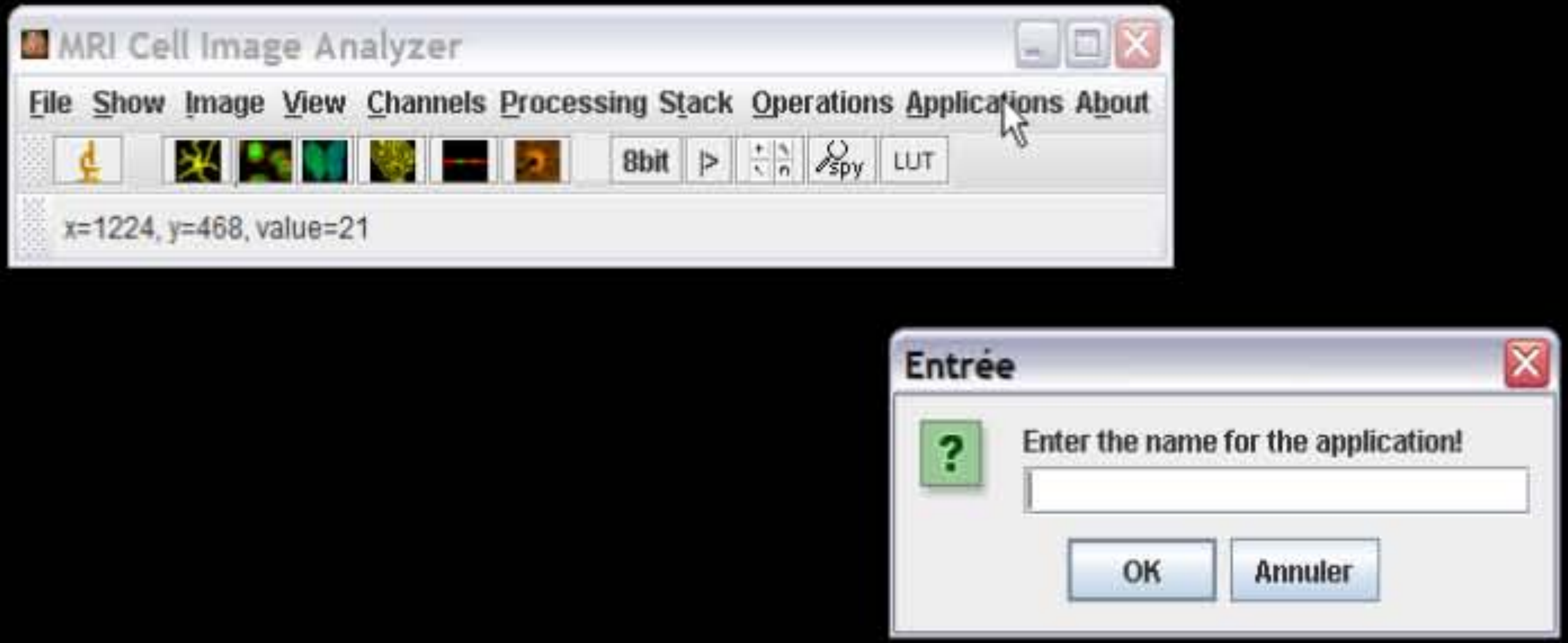
Back Next


MRI Cell Image Analyzer
File
Show
Image
View
Channels
Processing
Stack
Operations
Applications
About
8bit
spy
x=1224, y=468, value=21
new...
load...
install menu...
applications



Menu Applications->new...

Back Next

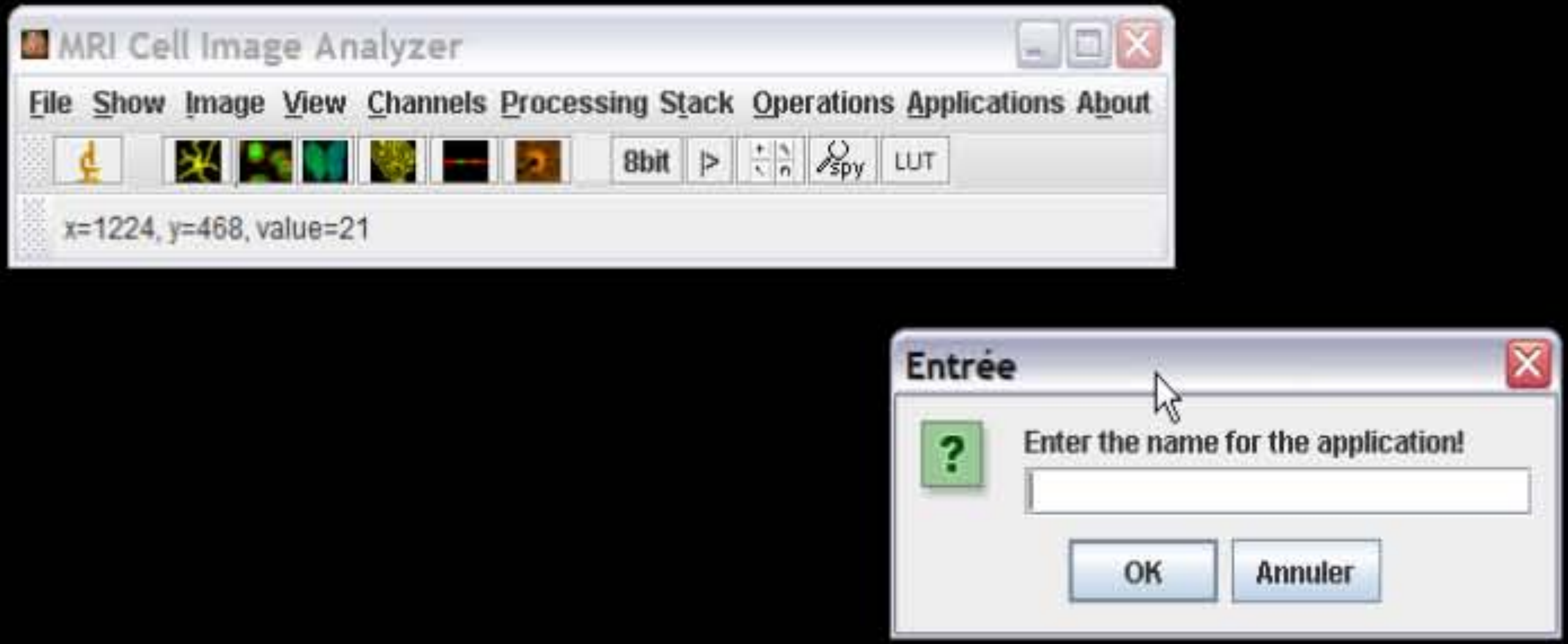

MRI Cell Image Analyzer
File Show Image View Channels Processing Stack Operations Applications About
8bit
LUT
Entrée
OK
Annuler

x=1224, y=468, value=21

Enter the name for the application!

Enter the name of the new application

Back Next

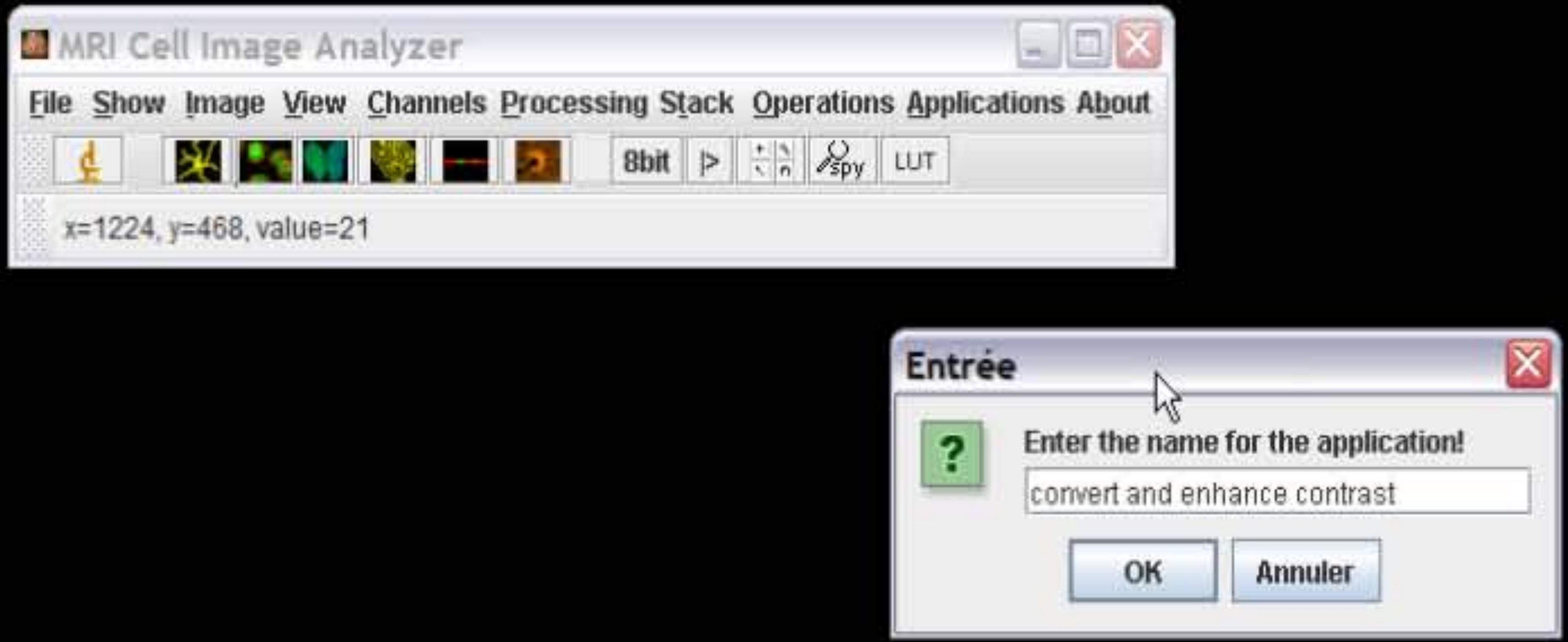

MRI Cell Image Analyzer
File Show Image View Channels Processing Stack Operations Applications About
8bit
LUT

x=1224, y=468, value=21

Entrée

Enter the name for the application!

Back Next

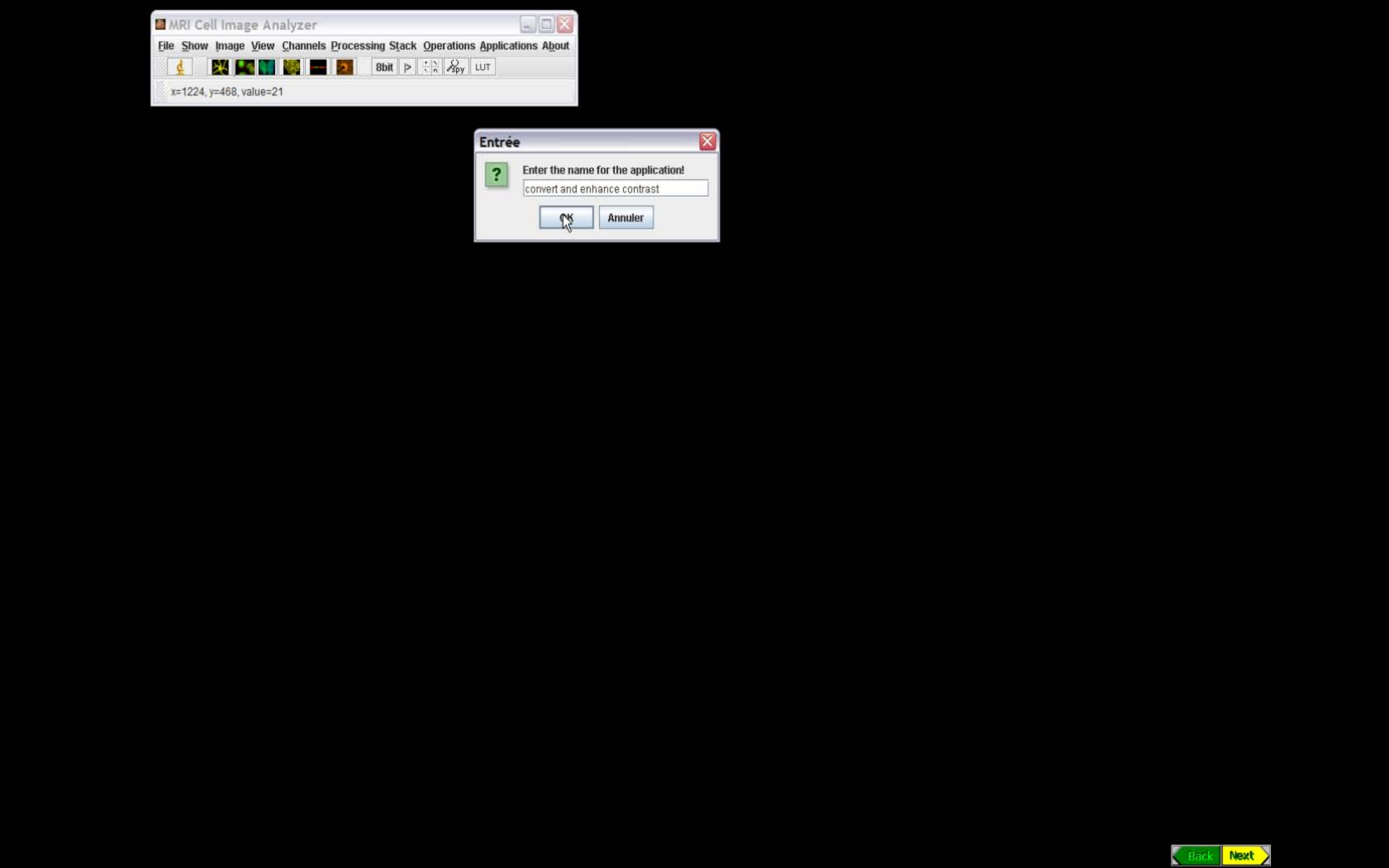

MRI Cell Image Analyzer
File Show Image View Channels Processing Stack Operations Applications About
8bit
LUT
spy
Entrée

x=1224, y=468, value=21

Enter the name for the application!

convert and enhance contrast

Back Next

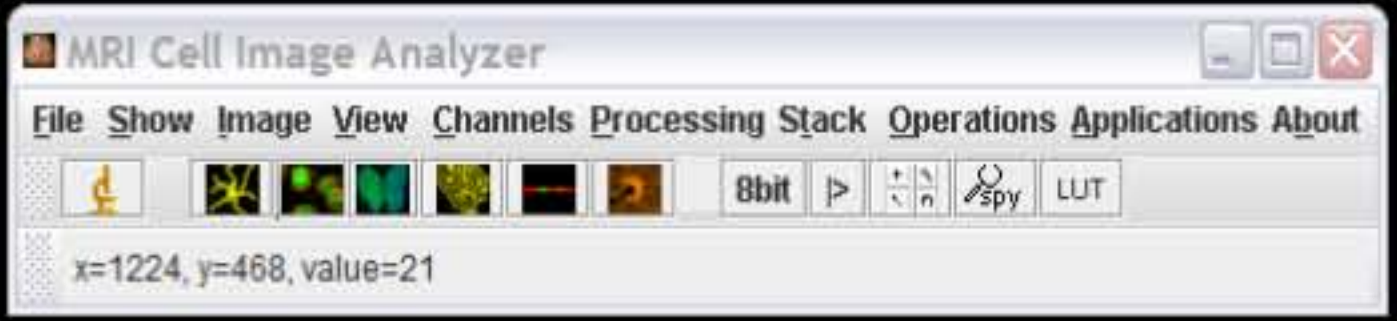


x=1224, y=468, value=21

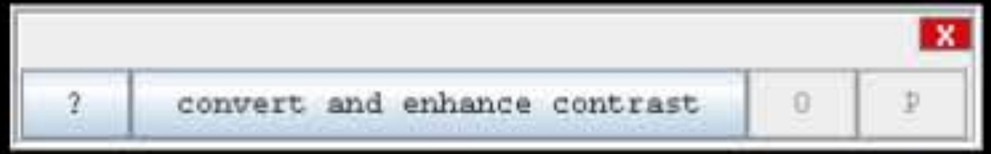
Entrée

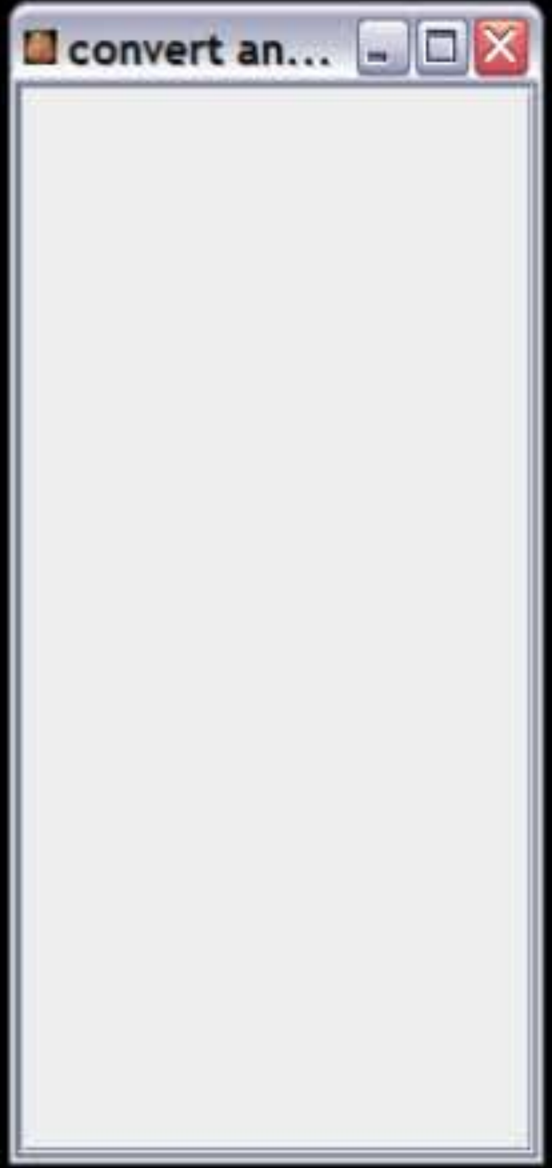
Enter the name for the application!

convert and enhance contrast


MRI Cell Image Analyzer
File Show Image View Channels Processing Stack Operations Applications About
8bit
LUT

x=1224, y=468, value=21

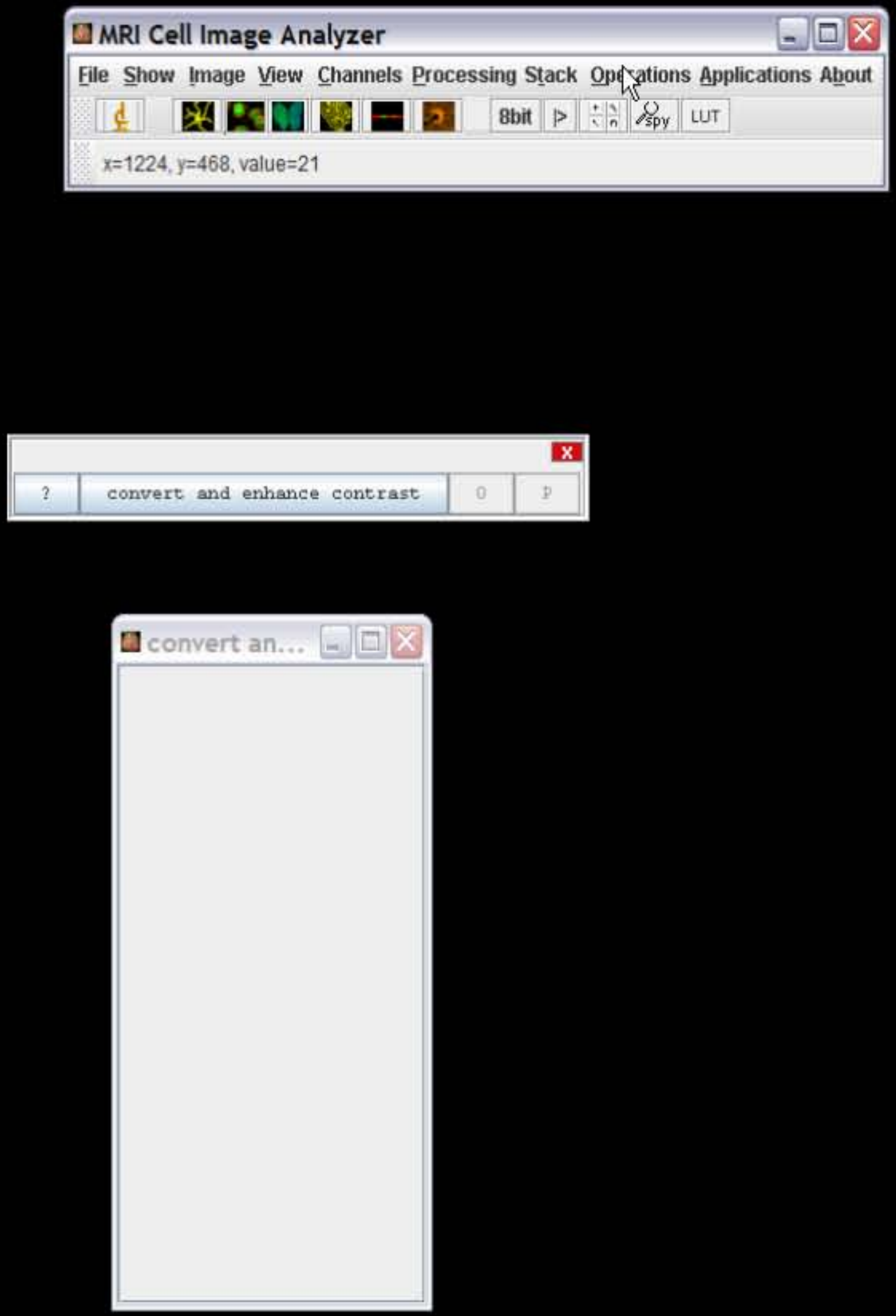




tile representation with buttons
- help
- start application

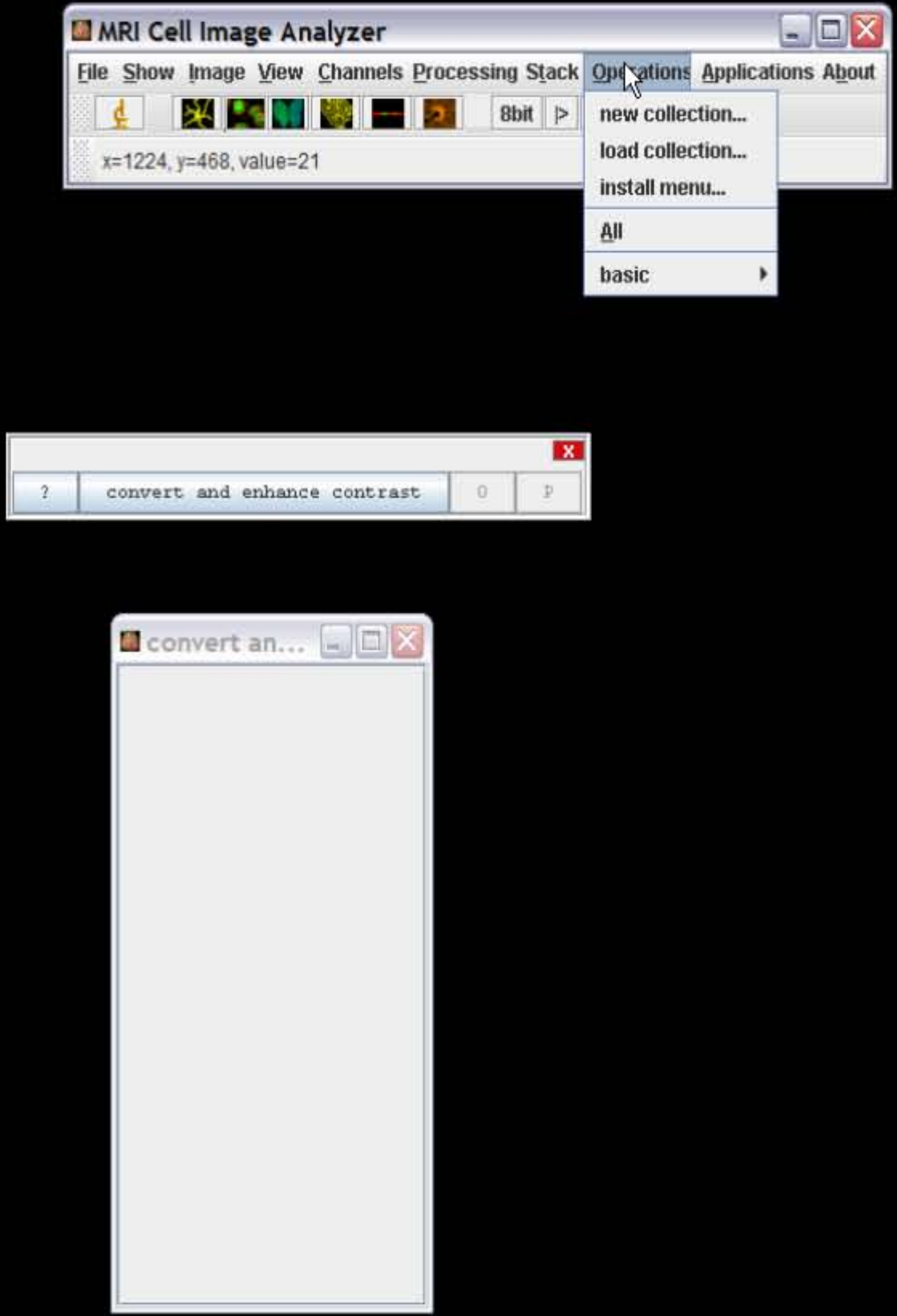
box representation
- list of operations (empty)

Back Next



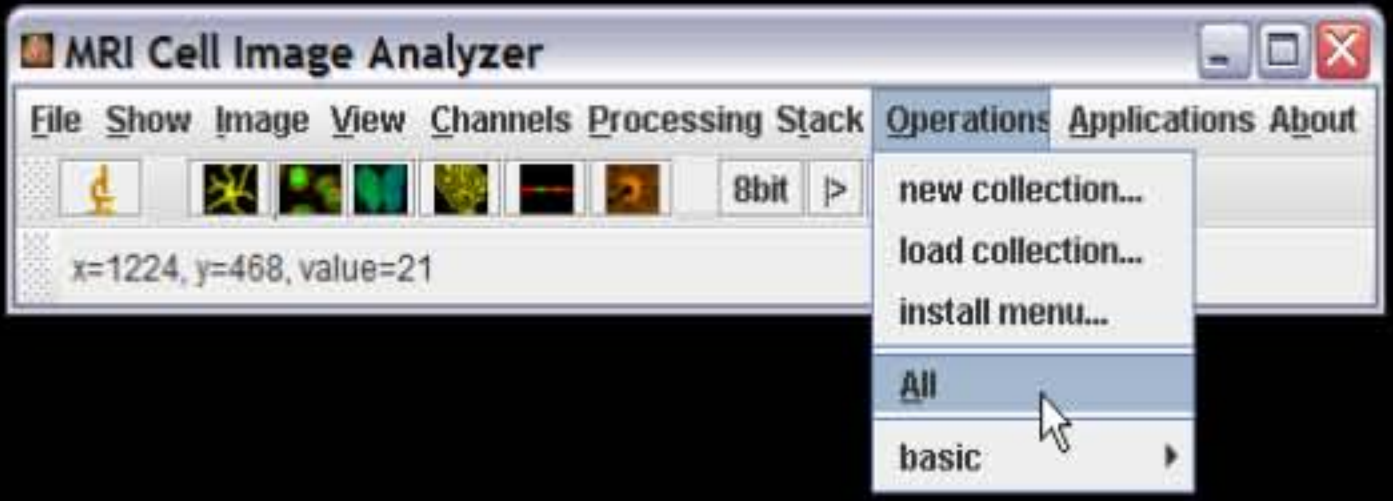
x=1224, y=468, value=21

Back Next

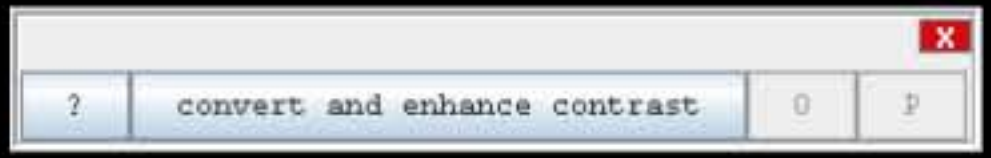


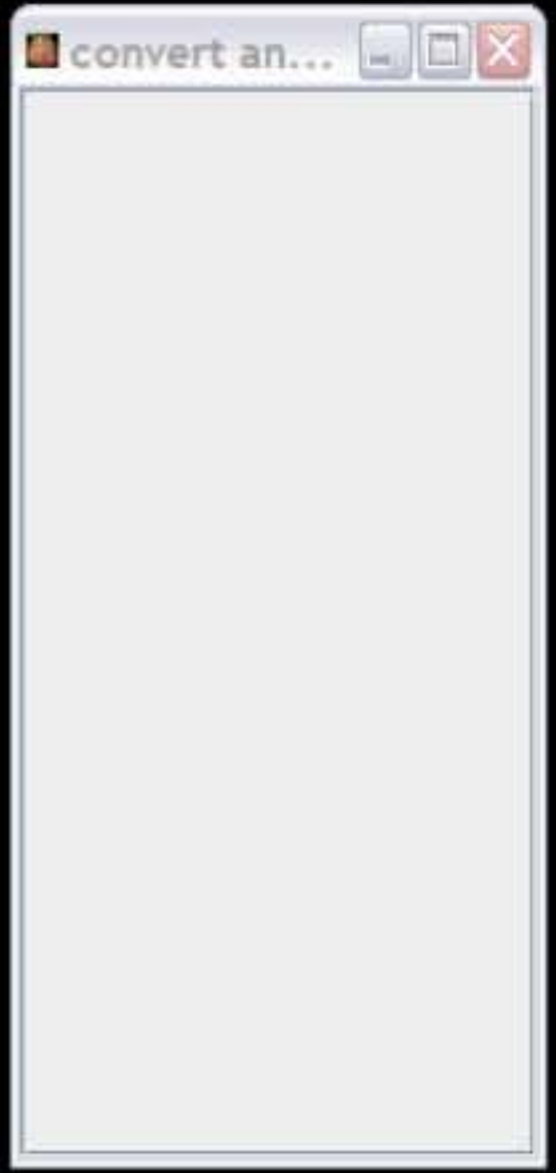
x=1224, y=468, value=21

Back Next



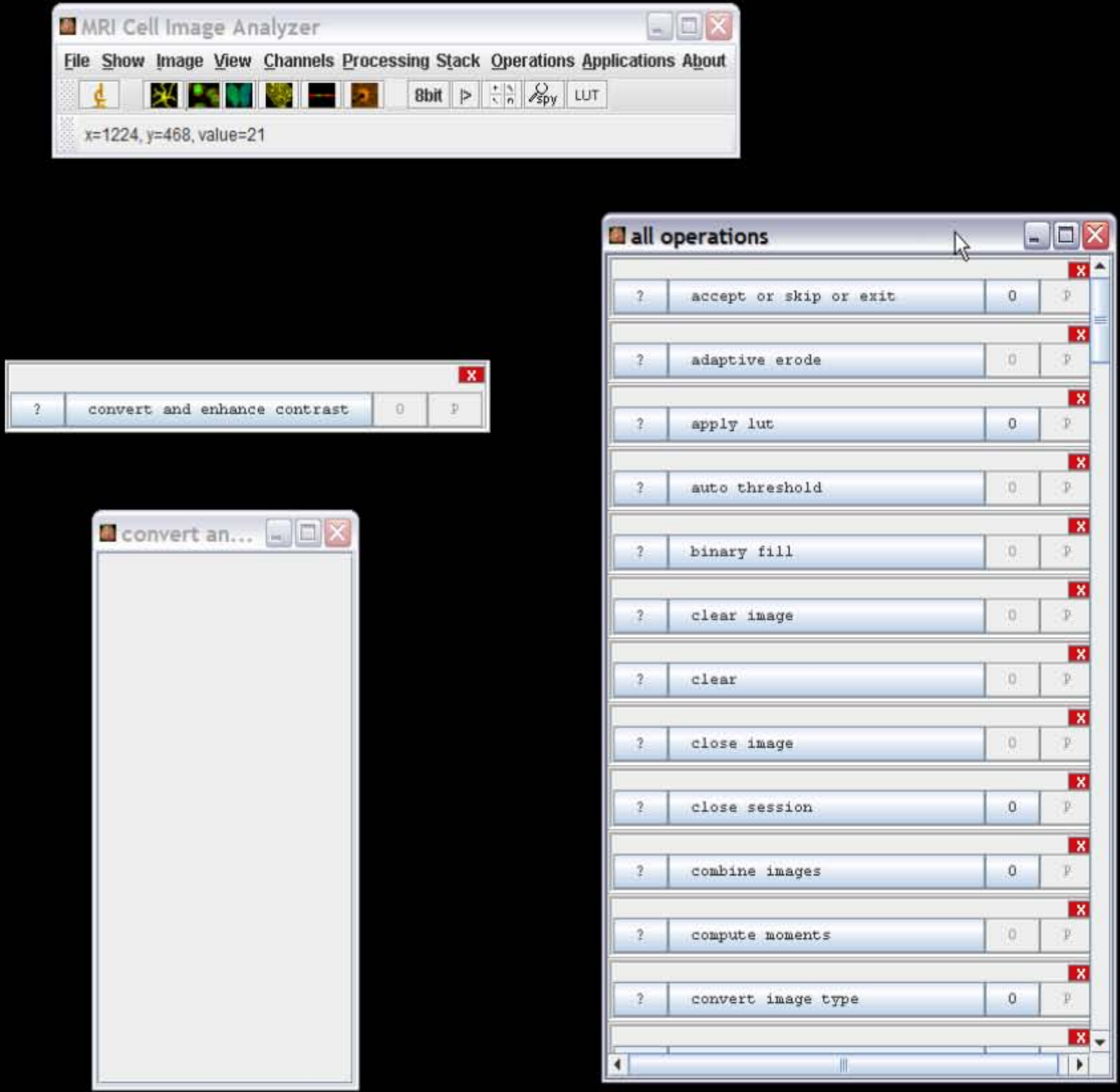
x=1224, y=468, value=21





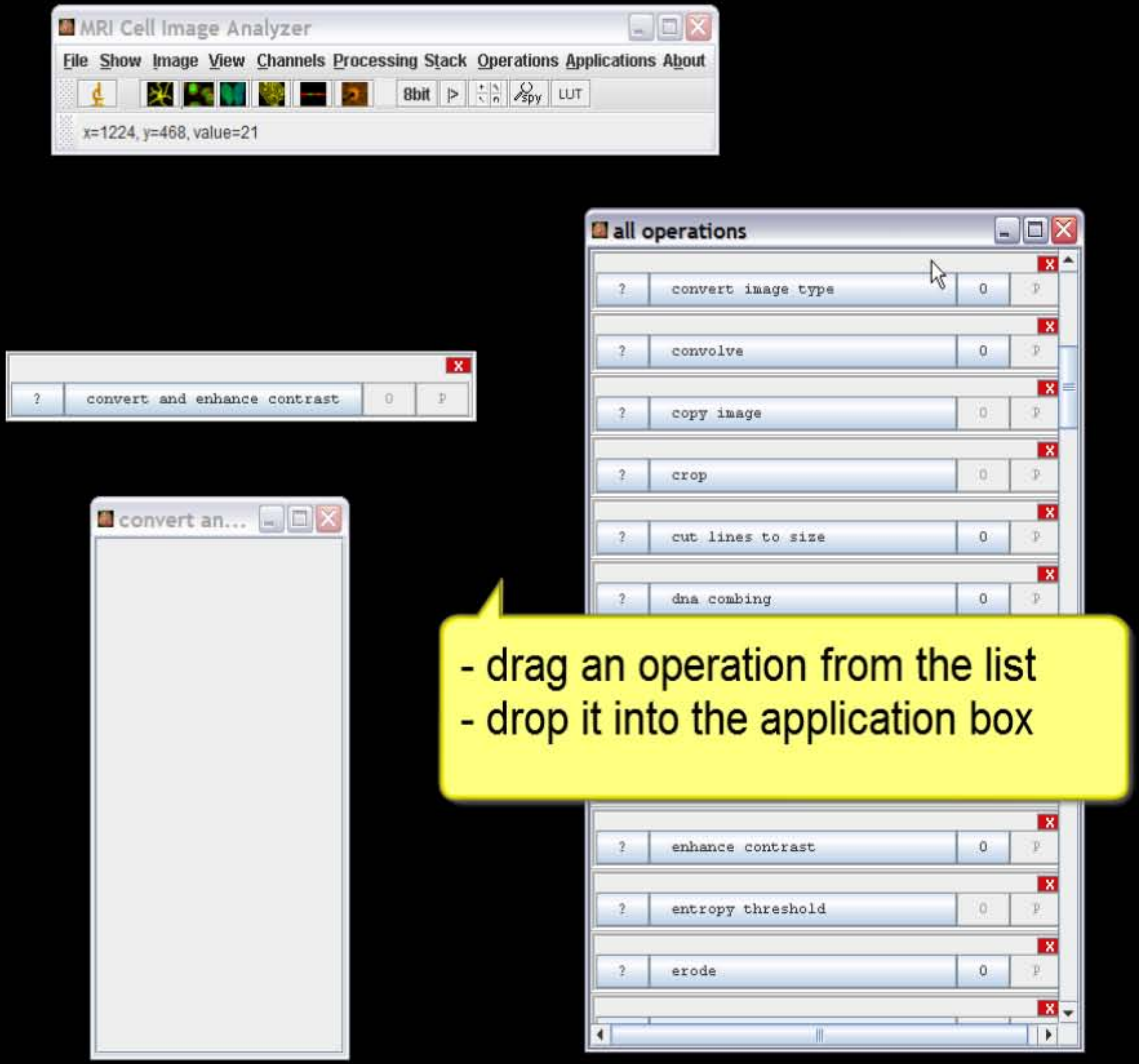
to add operations, open
- collection of operations
or
- list of all operations

Back Next



x=1224, y=468, value=21

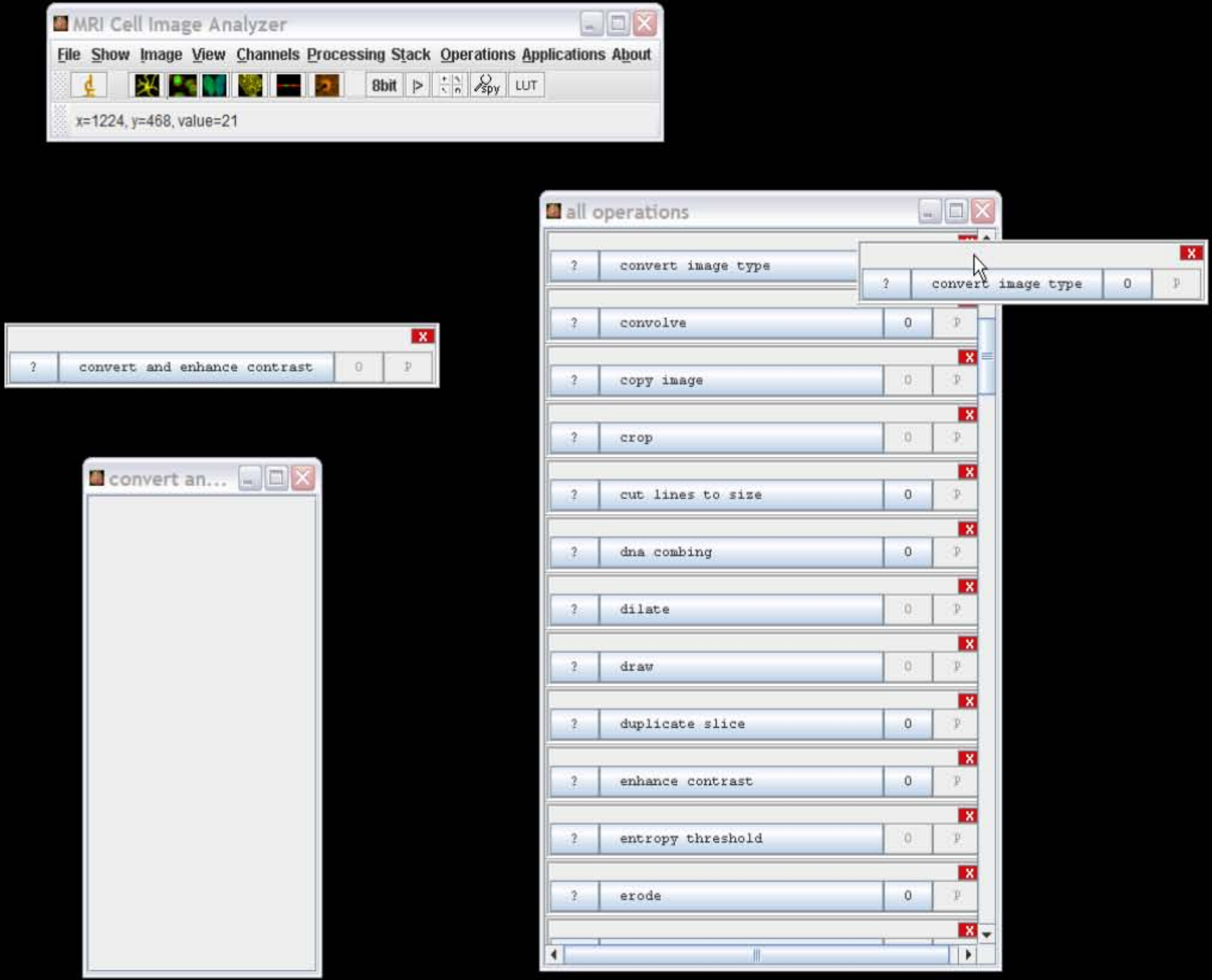
Back Next



```
x=1224, y=468, value=21
```

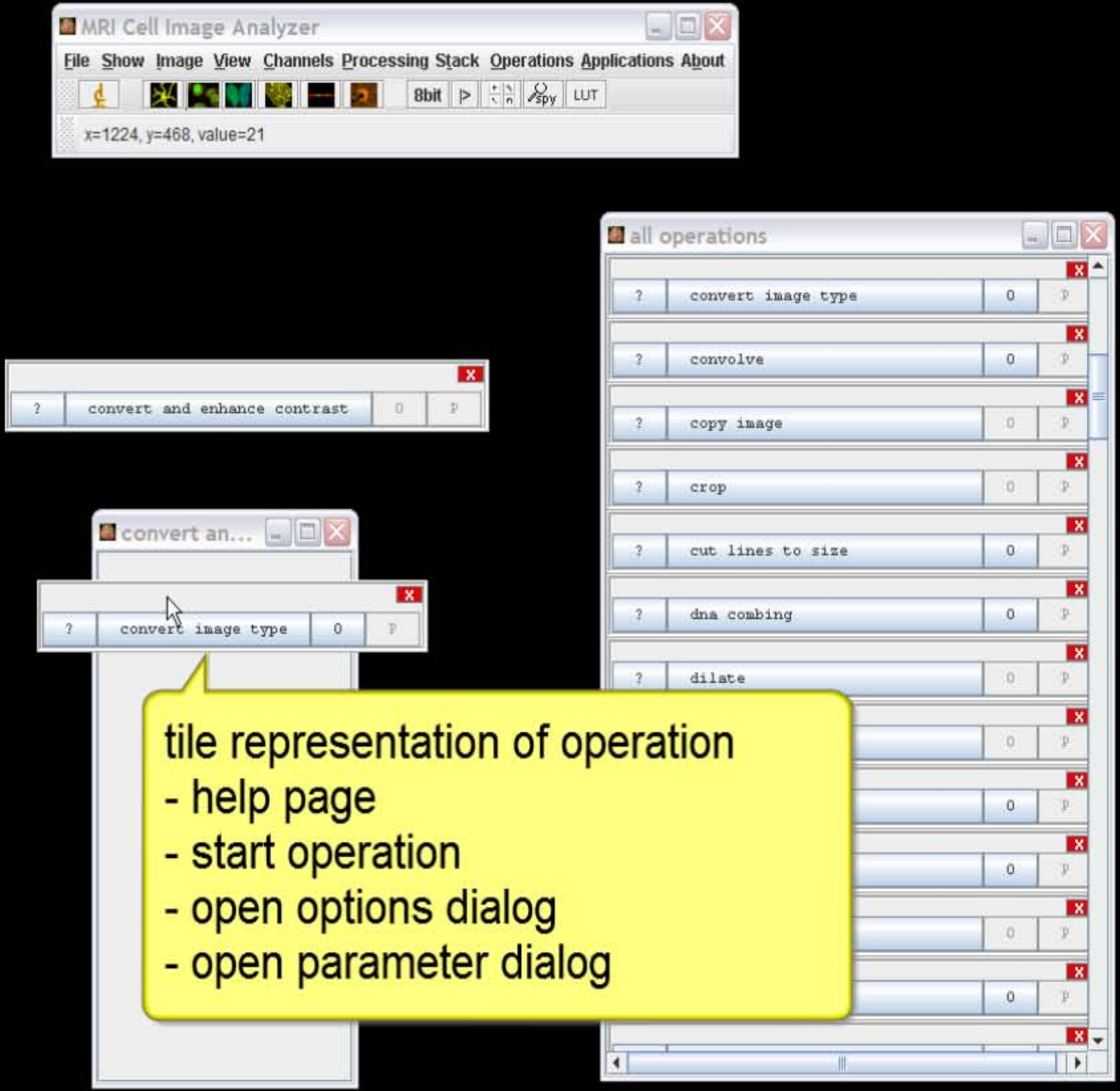
- drag an operation from the list
- drop it into the application box

Back Next



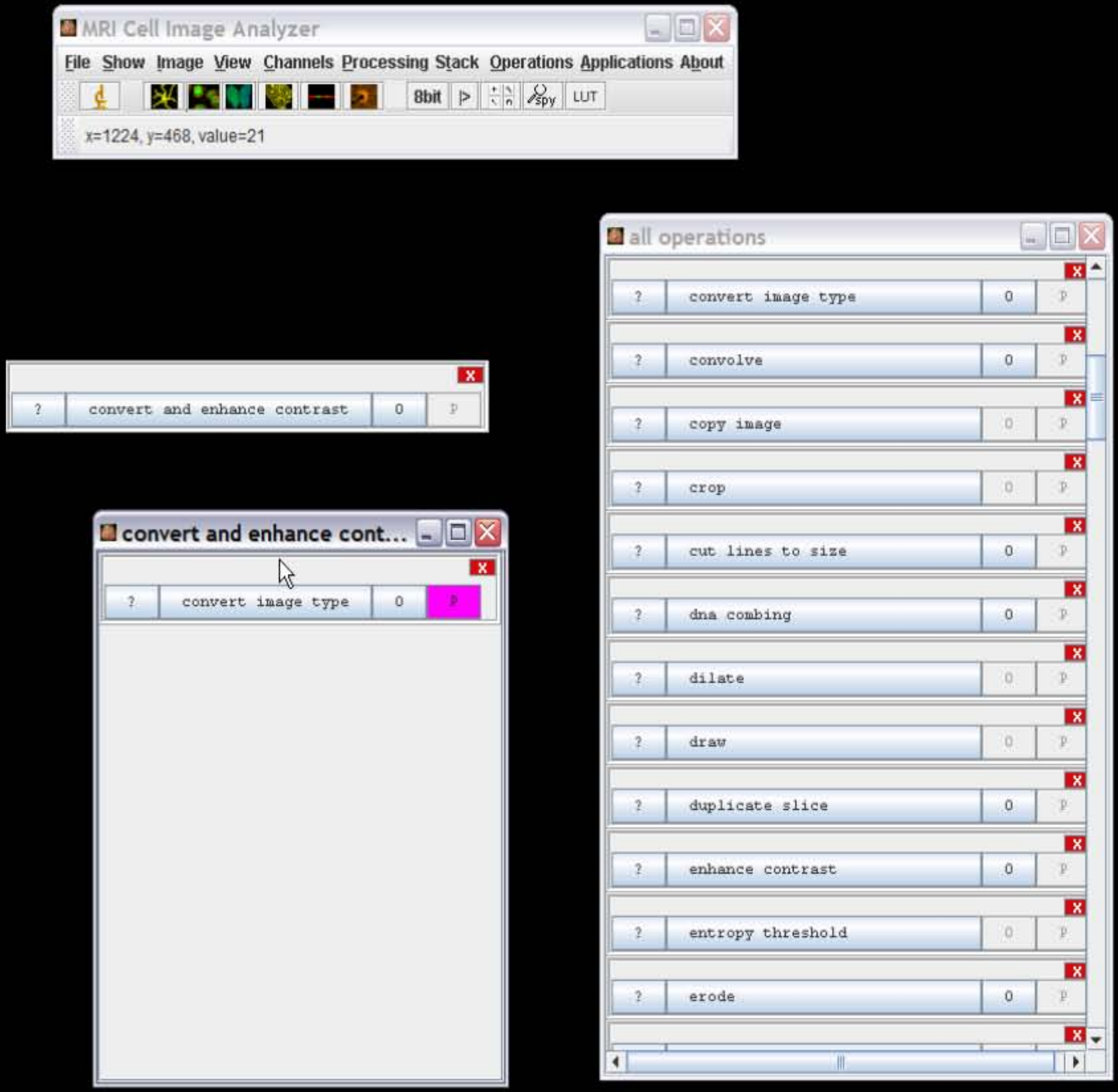
x=1224, y=468, value=21

Back Next

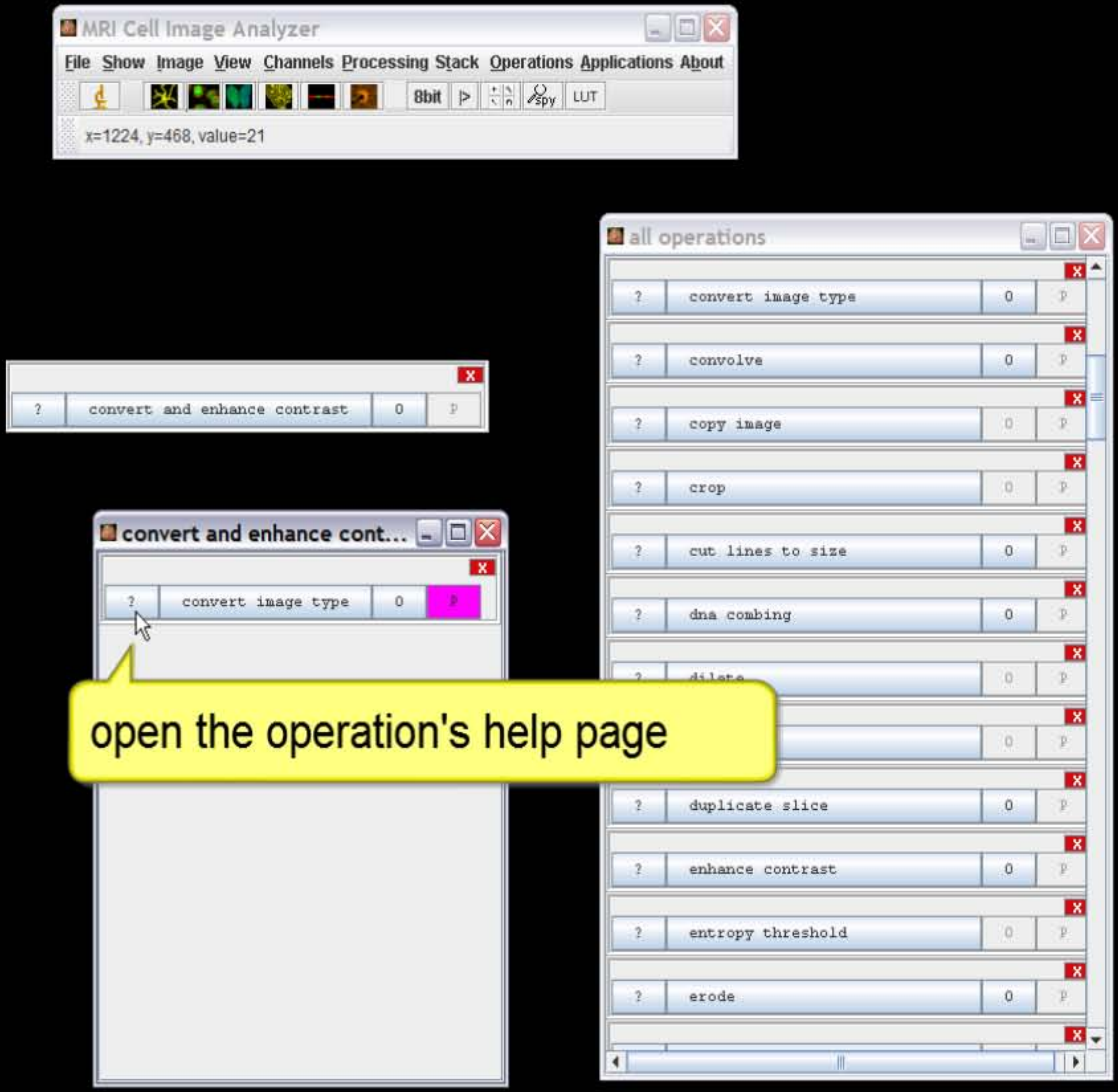


tile representation of operation
- help page
- start operation
- open options dialog
- open parameter dialog

Back Next

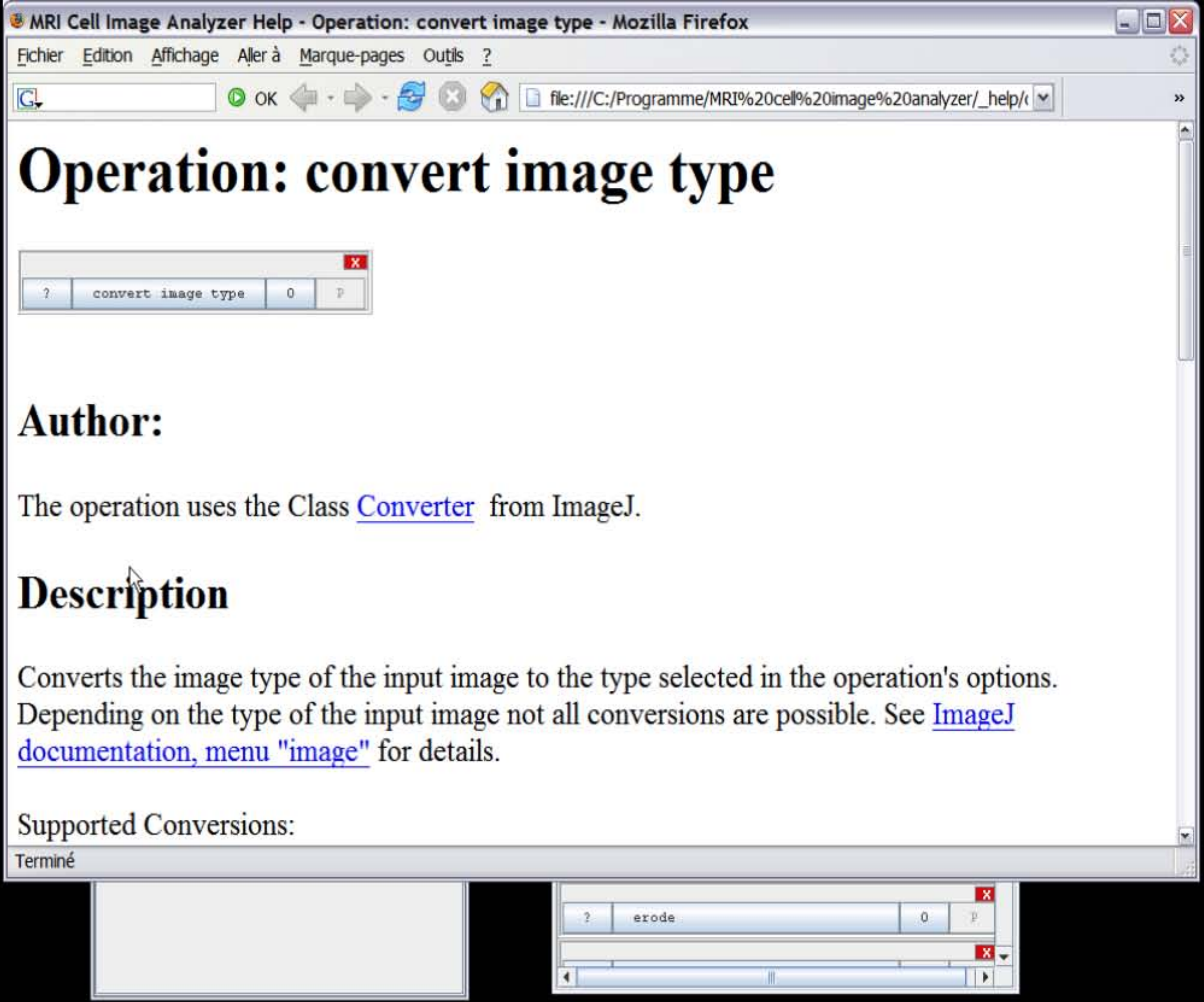

MRI Cell Image Analyzer
File Show Image View Channels Processing Stack Operations Applications About
8bit
LUT
x=1224, y=468, value=21
? convert and enhance contrast 0 P
convert and enhance cont...
? convert image type 0 P
all operations
? convert image type 0 P
? convolve 0 P
? copy image 0 P
? crop 0 P
? cut lines to size 0 P
? dna combing 0 P
? dilate 0 P
? draw 0 P
? duplicate slice 0 P
? enhance contrast 0 P
? entropy threshold 0 P
? erode 0 P

Back Next



x=1224, y=468, value=21

open the operation's help page



MRI Cell Image Analyzer Help - Operation: convert image type - Mozilla Firefox
Fichier
Edition
Affichage
Aller à
Marque-pages
Outils
?
OK
file:///C:/Programme/MRI%20cell%20image%20analyzer/_help/(
?
convert image type
0
P
Terminé
?
erode
0
P

Operation: convert image type

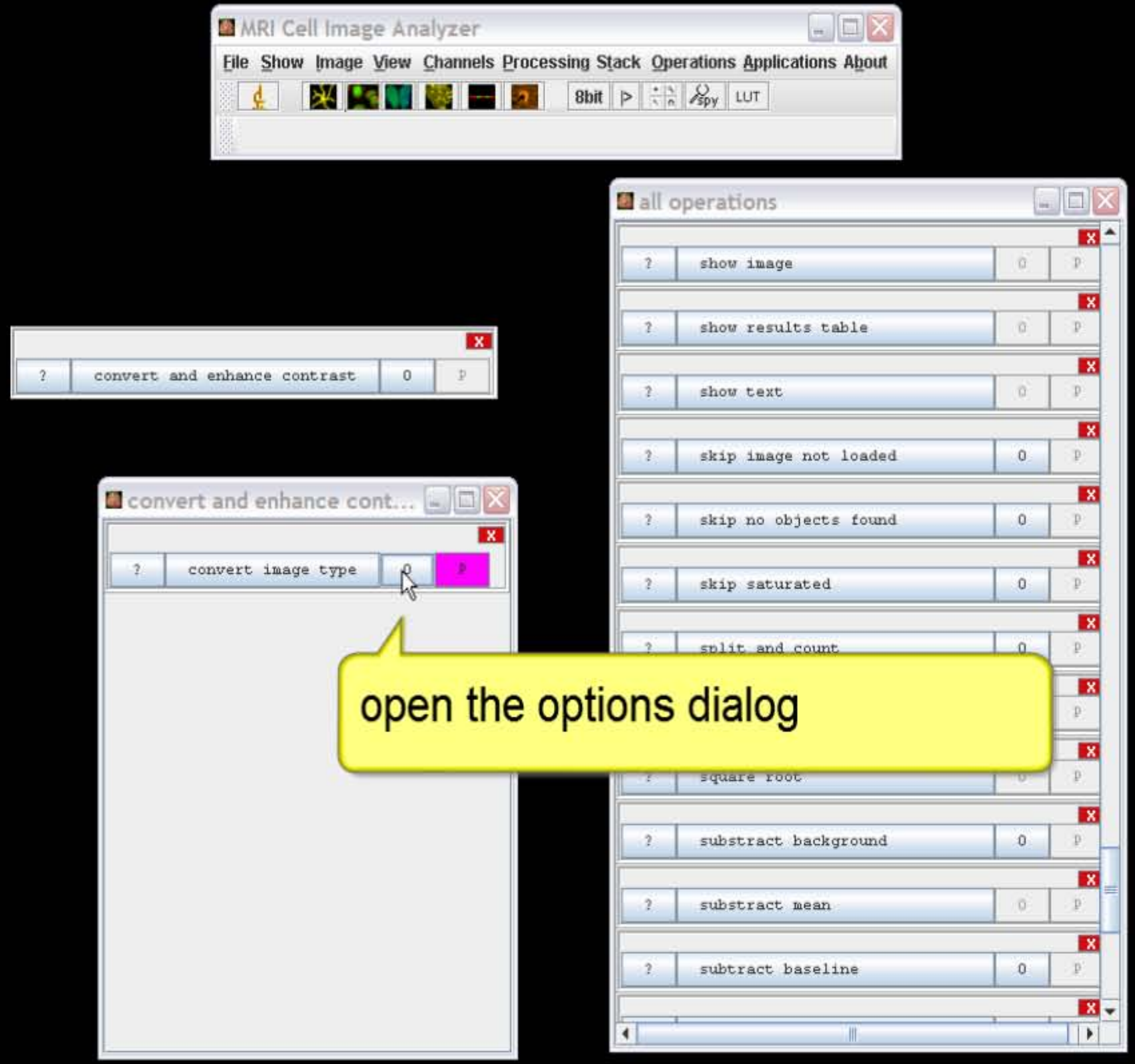
Author:

The operation uses the Class Converter from ImageJ.

Description

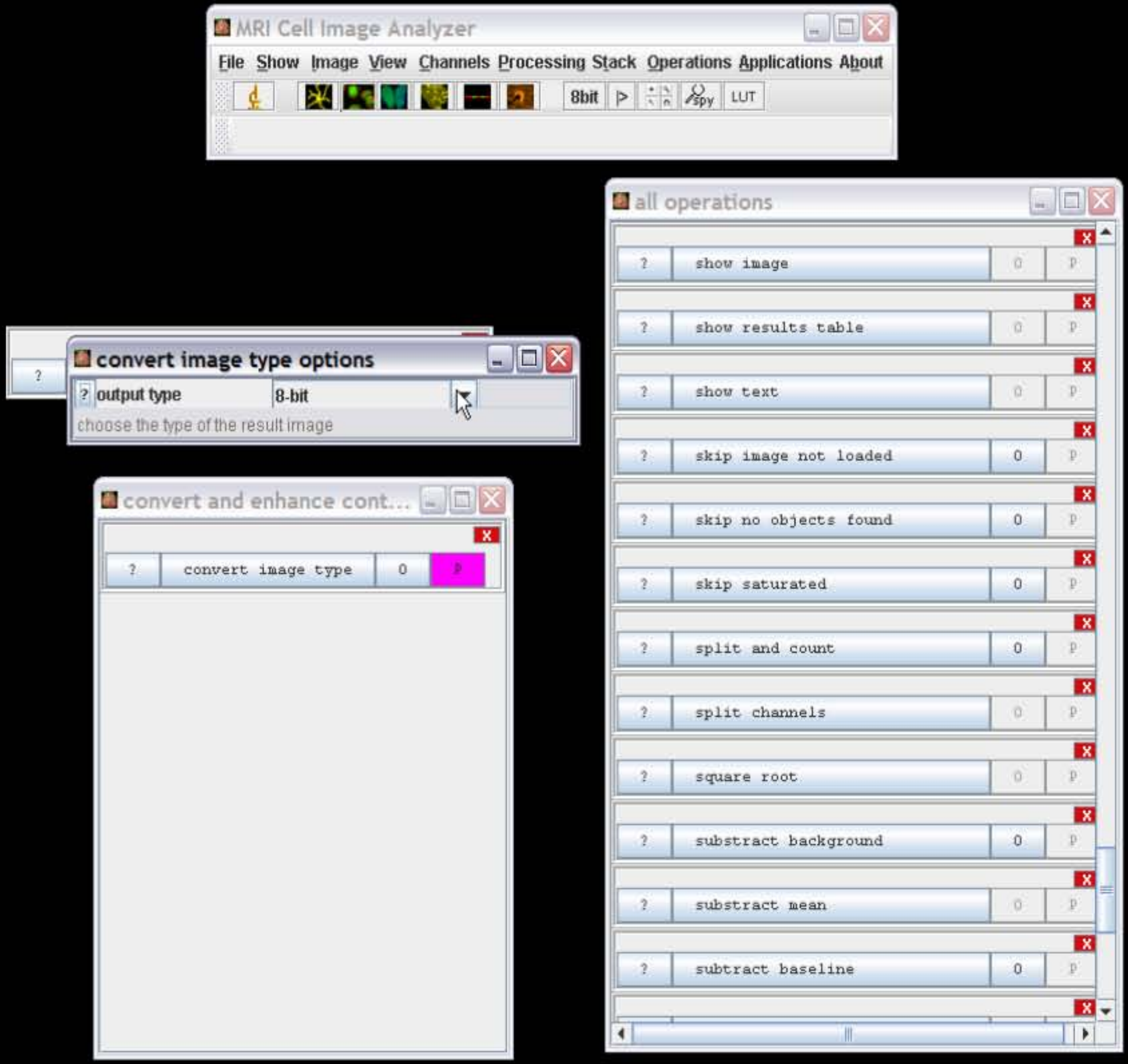
Converts the image type of the input image to the type selected in the operation's options. Depending on the type of the input image not all conversions are possible. See ImageJ documentation, menu "image" for details.

Supported Conversions:



open the options dialog

Back Next

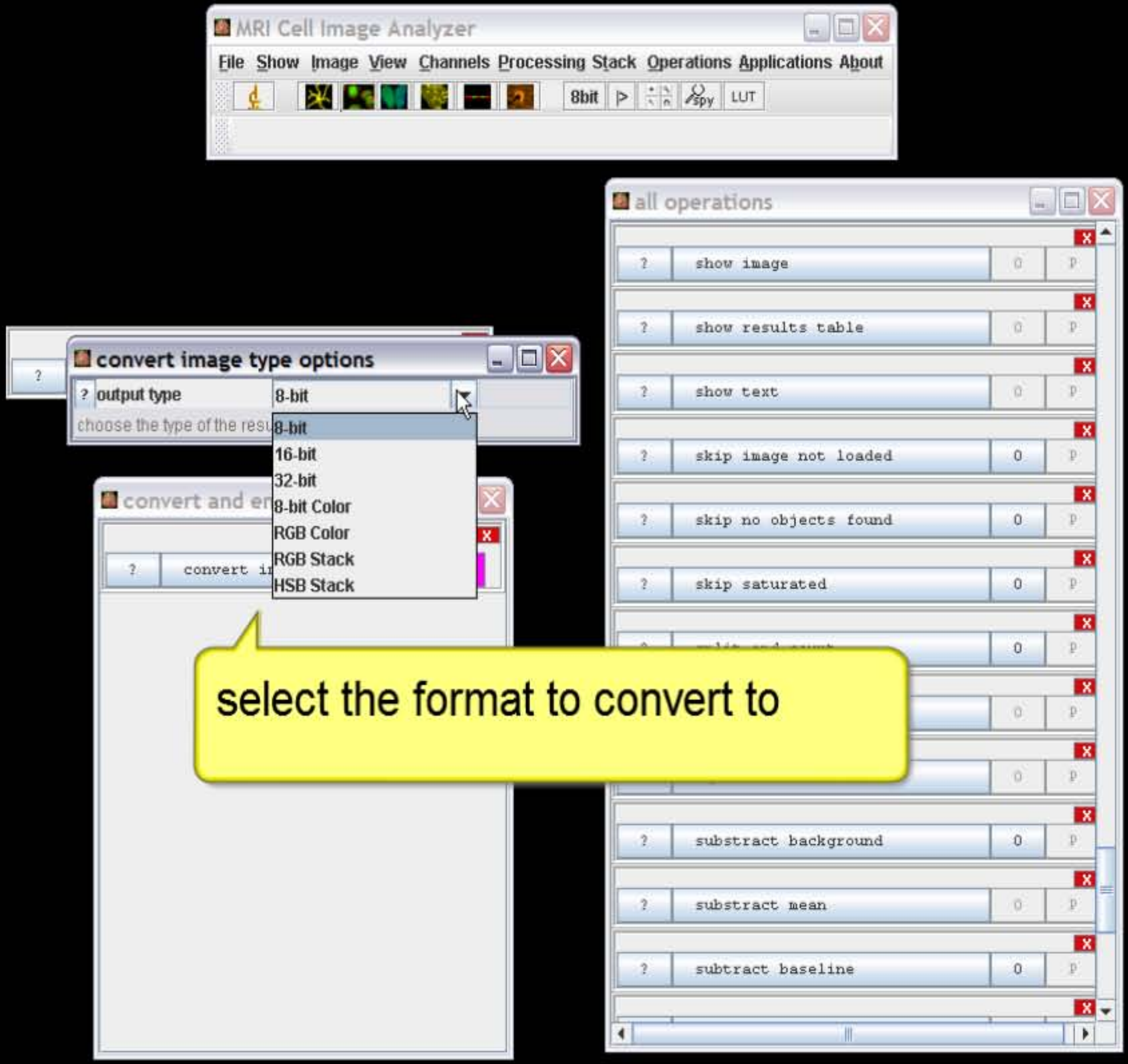


convert image type options

? output type 8-bit

choose the type of the result image

Back Next



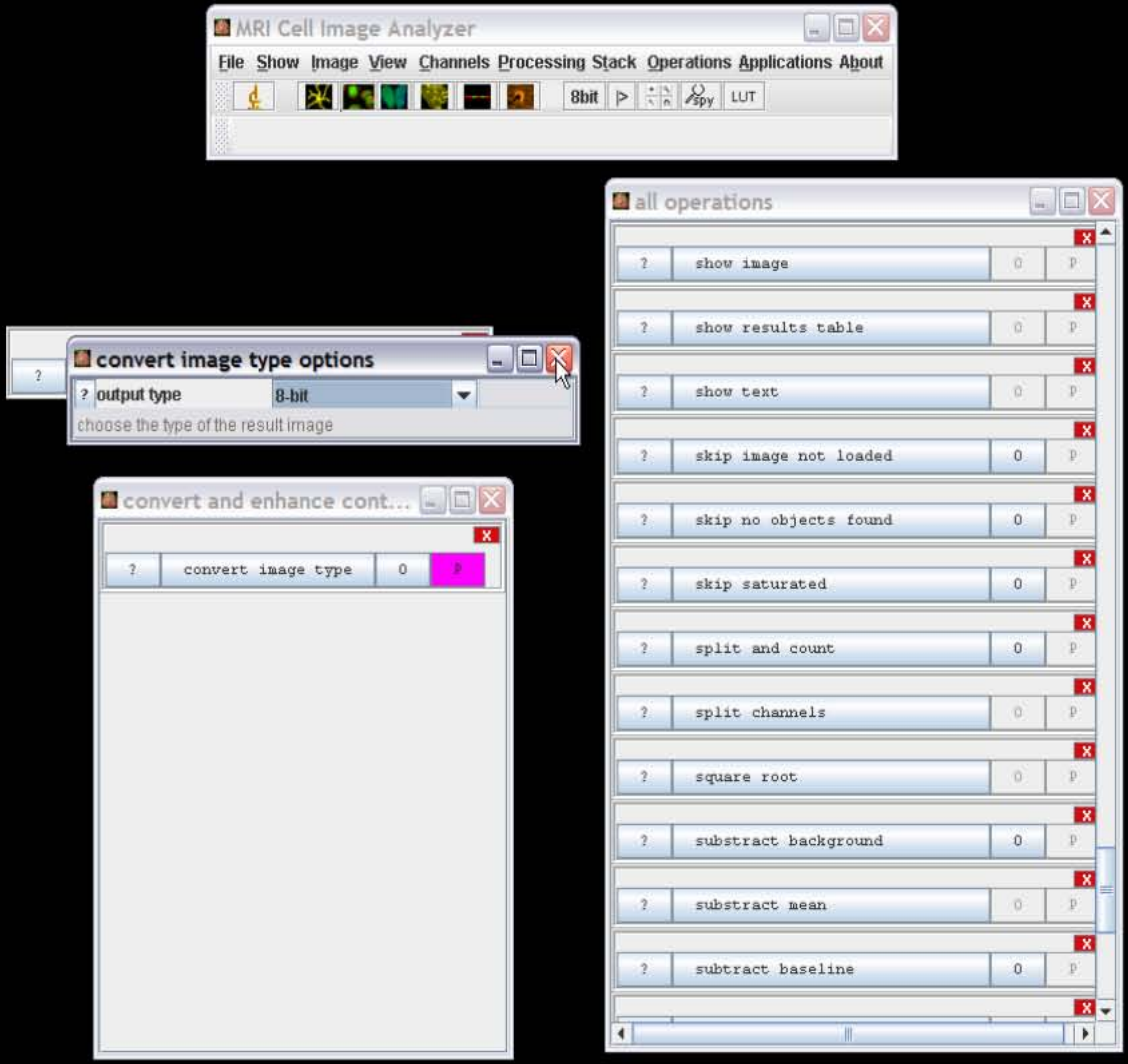
convert image type options

output type: 8-bit

choose the type of the resu

select the format to convert to

Back Next


MRI Cell Image Analyzer
File Show Image View Channels Processing Stack Operations Applications About
8bit
LUT

convert image type options

output type: 8-bit

choose the type of the result image

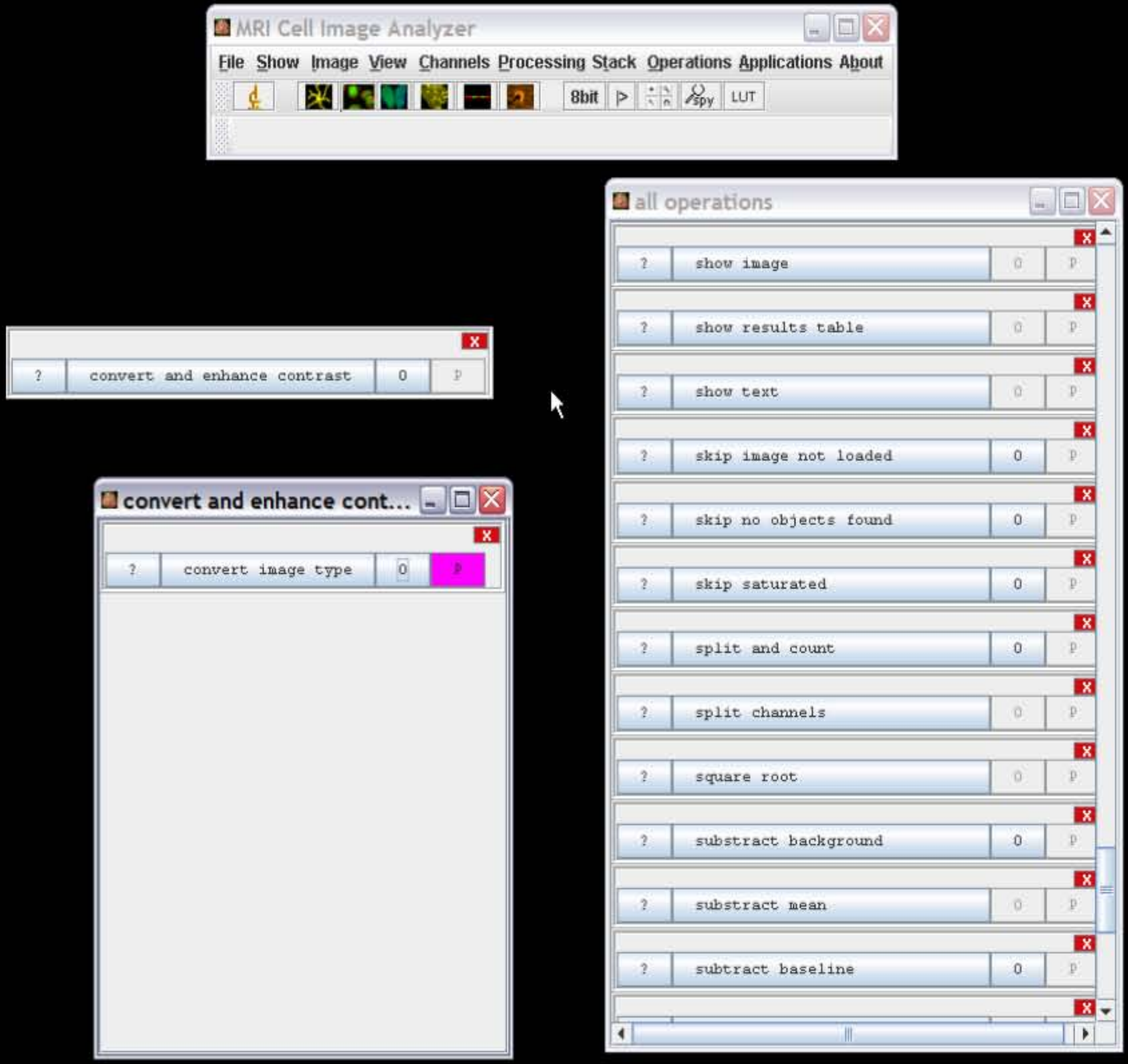
convert and enhance cont...

?	convert image type	0	P

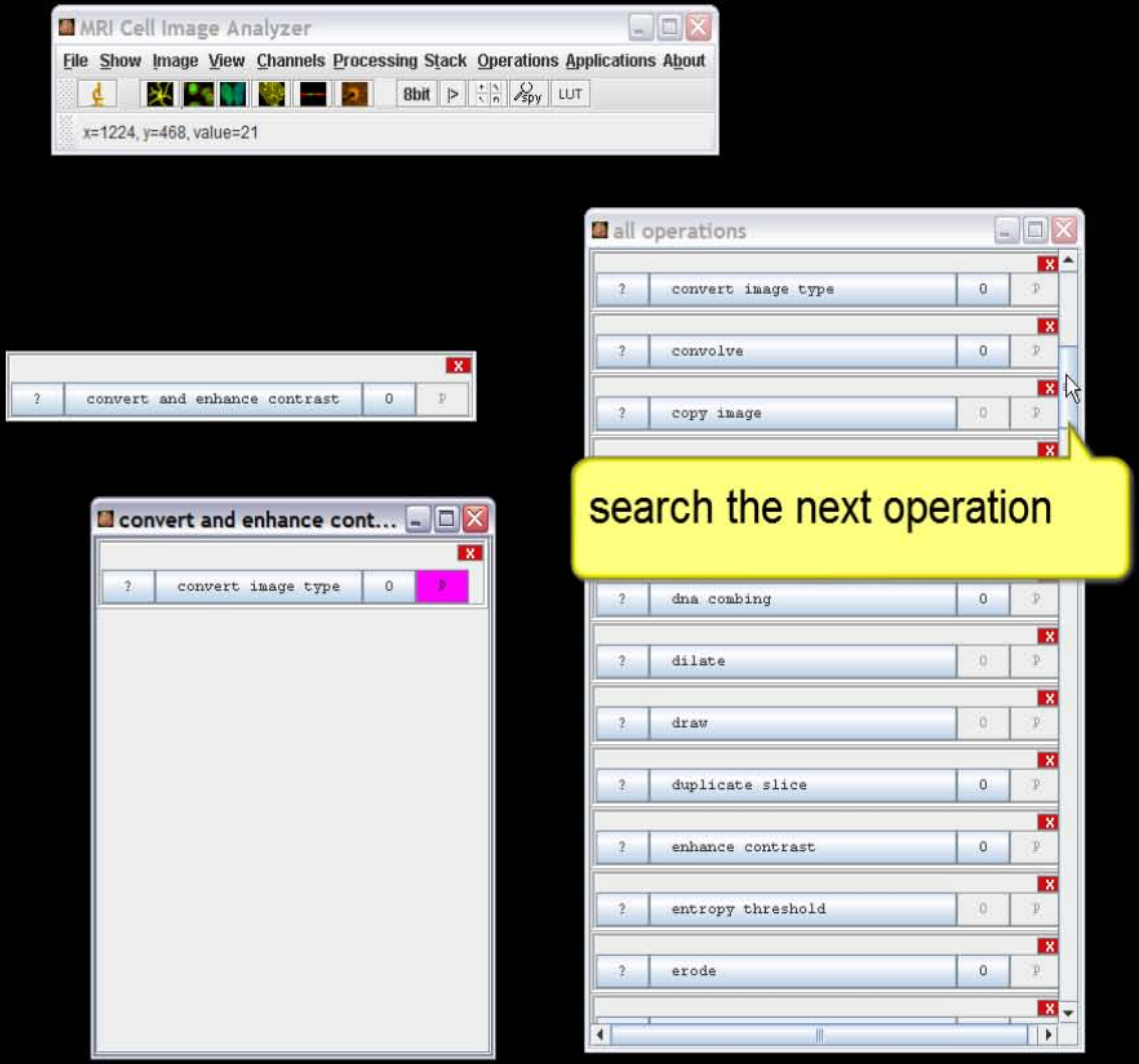
all operations

?	show image	0	P
?	show results table	0	P
?	show text	0	P
?	skip image not loaded	0	P
?	skip no objects found	0	P
?	skip saturated	0	P
?	split and count	0	P
?	split channels	0	P
?	square root	0	P
?	substract background	0	P
?	substract mean	0	P
?	subtract baseline	0	P

Back Next

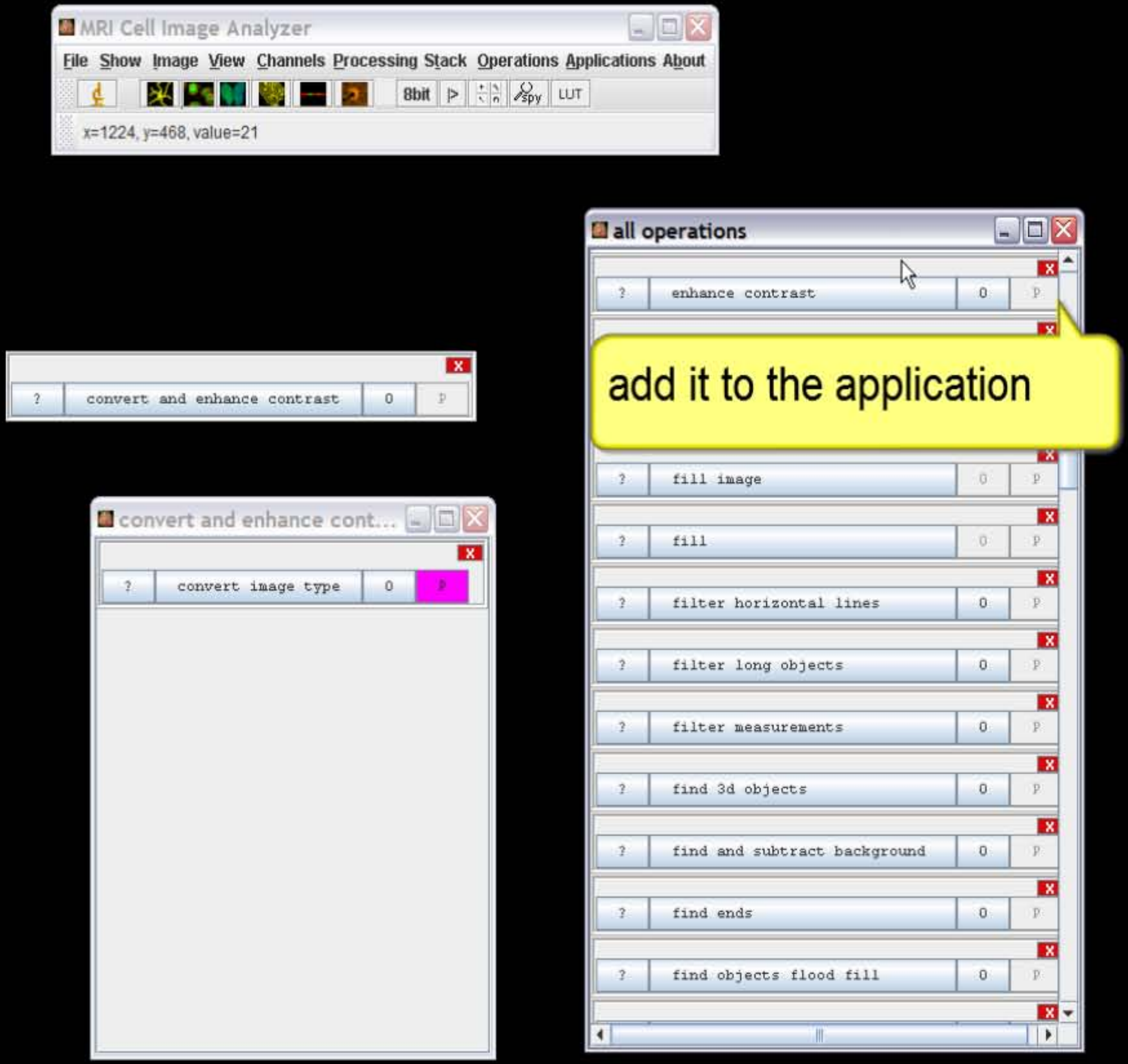

MRI Cell Image Analyzer
File Show Image View Channels Processing Stack Operations Applications About
8bit
LUT
? convert and enhance contrast 0 P
convert and enhance cont...
? convert image type 0 P
all operations
? show image 0 P
? show results table 0 P
? show text 0 P
? skip image not loaded 0 P
? skip no objects found 0 P
? skip saturated 0 P
? split and count 0 P
? split channels 0 P
? square root 0 P
? substract background 0 P
? substract mean 0 P
? subtract baseline 0 P

Back Next



search the next operation

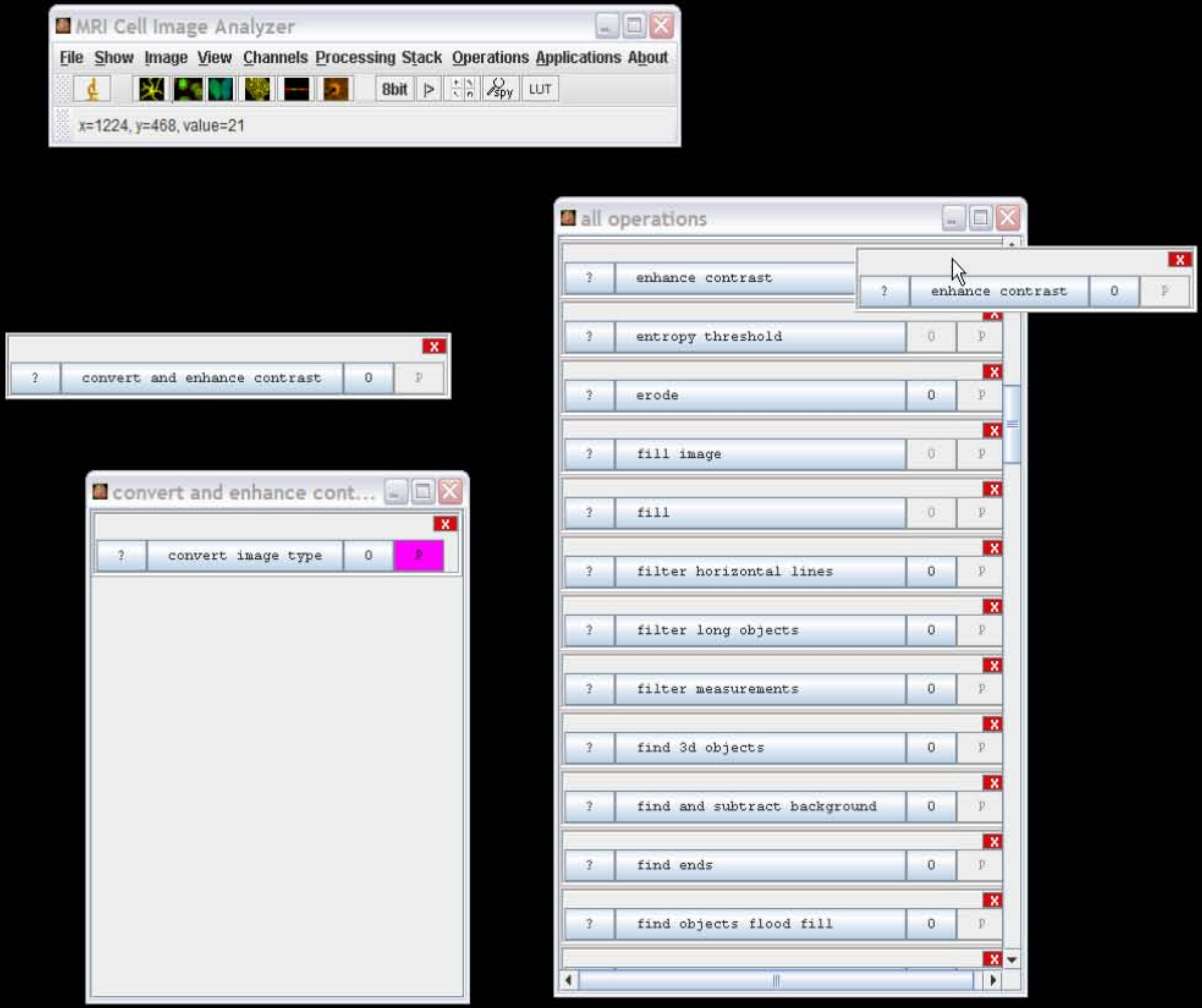
Back Next


MRI Cell Image Analyzer
File Show Image View Channels Processing Stack Operations Applications About
8bit
LUT
all operations
? enhance contrast 0 P
? fill image 0 P
? fill 0 P
? filter horizontal lines 0 P
? filter long objects 0 P
? filter measurements 0 P
? find 3d objects 0 P
? find and subtract background 0 P
? find ends 0 P
? find objects flood fill 0 P
? convert and enhance contrast 0 P
convert and enhance cont...
? convert image type 0 P

x=1224, y=468, value=21

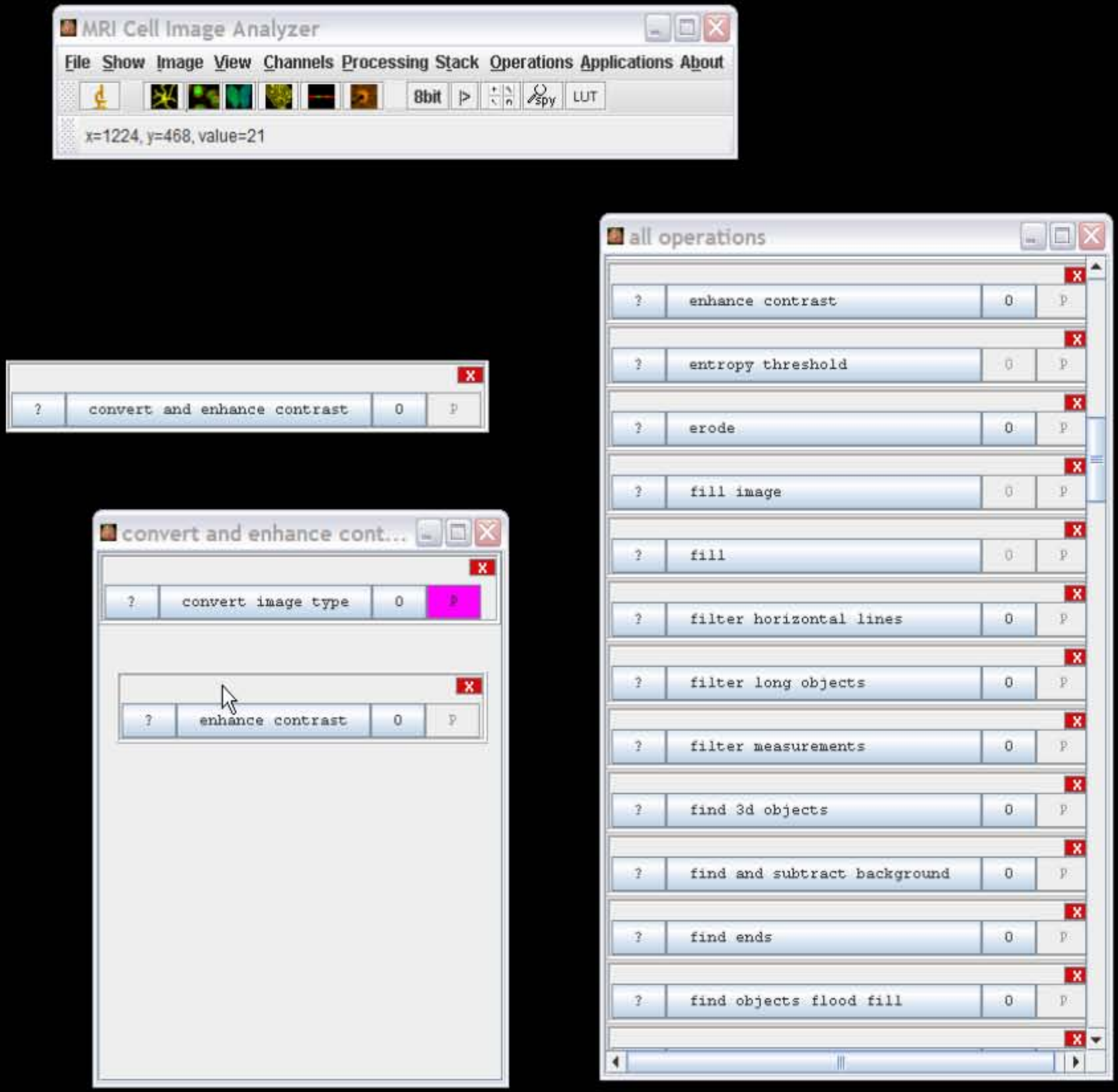
add it to the application

Back Next



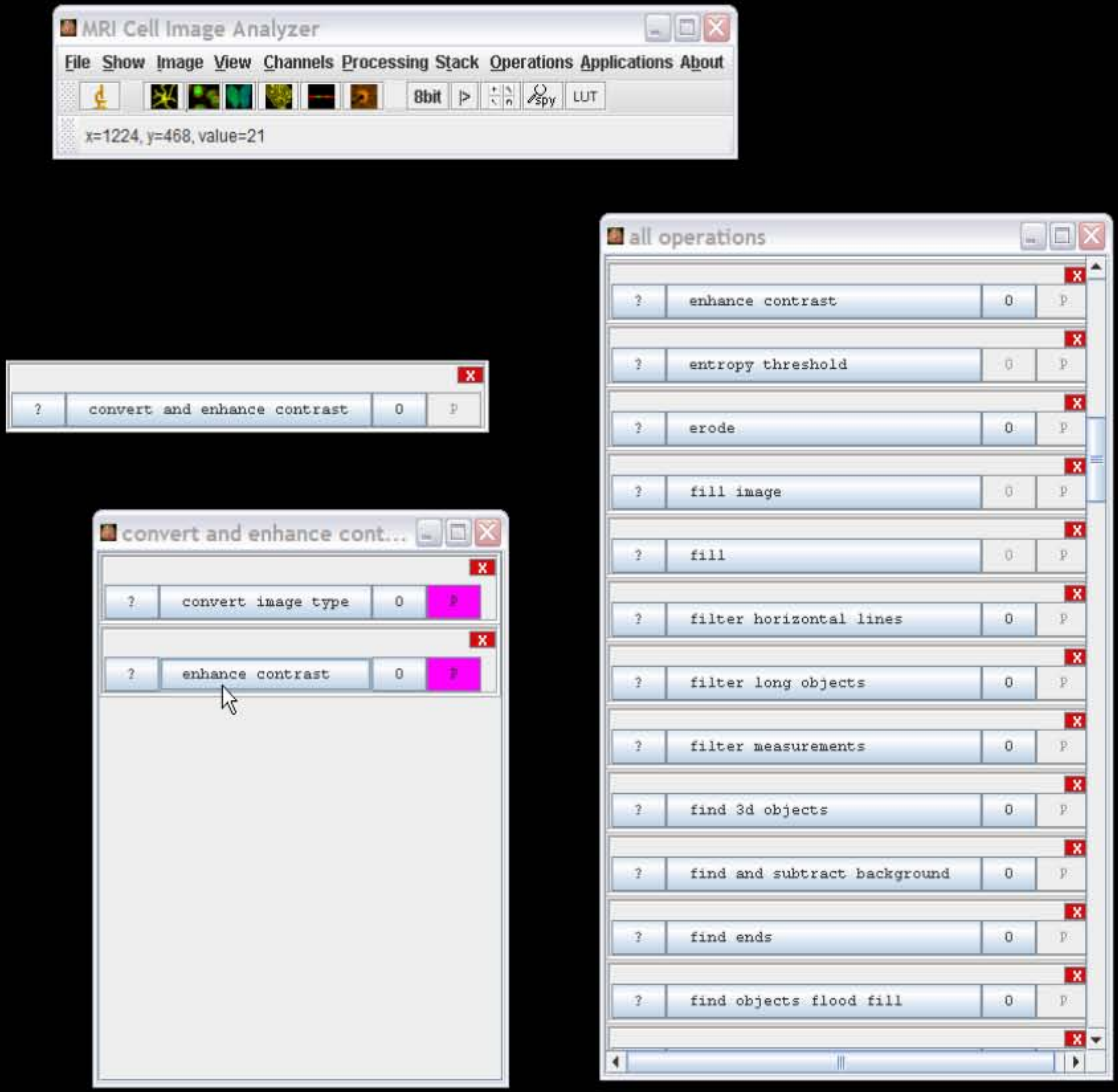
x=1224, y=468, value=21

Back Next


MRI Cell Image Analyzer
File Show Image View Channels Processing Stack Operations Applications About
8bit
LUT
all operations
? enhance contrast 0 P
? entropy threshold 0 P
? erode 0 P
? fill image 0 P
? fill 0 P
? filter horizontal lines 0 P
? filter long objects 0 P
? filter measurements 0 P
? find 3d objects 0 P
? find and subtract background 0 P
? find ends 0 P
? find objects flood fill 0 P
? convert and enhance contrast 0 P
convert and enhance cont...
? convert image type 0 P
? enhance contrast 0 P

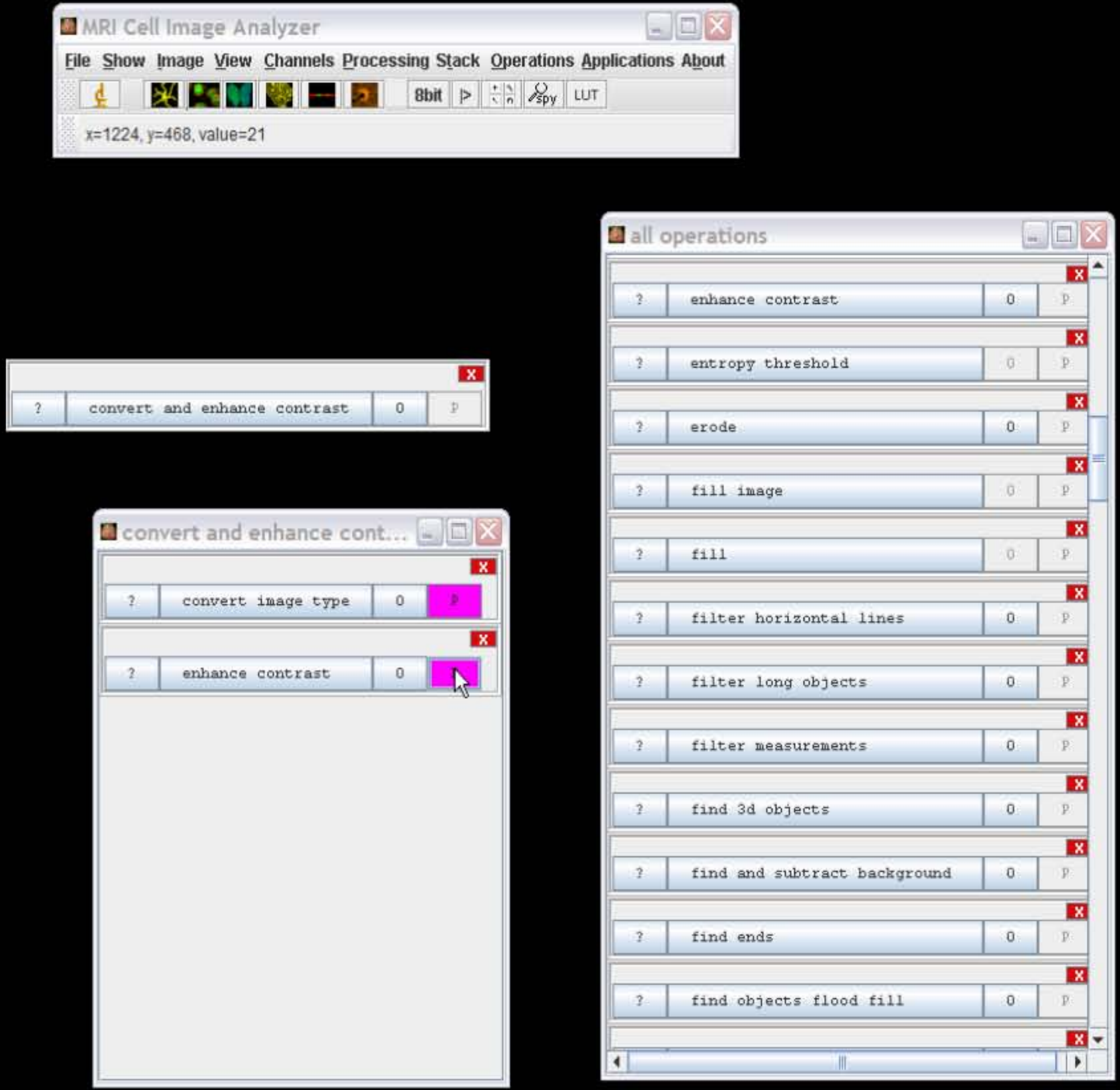
x=1224, y=468, value=21

Back Next



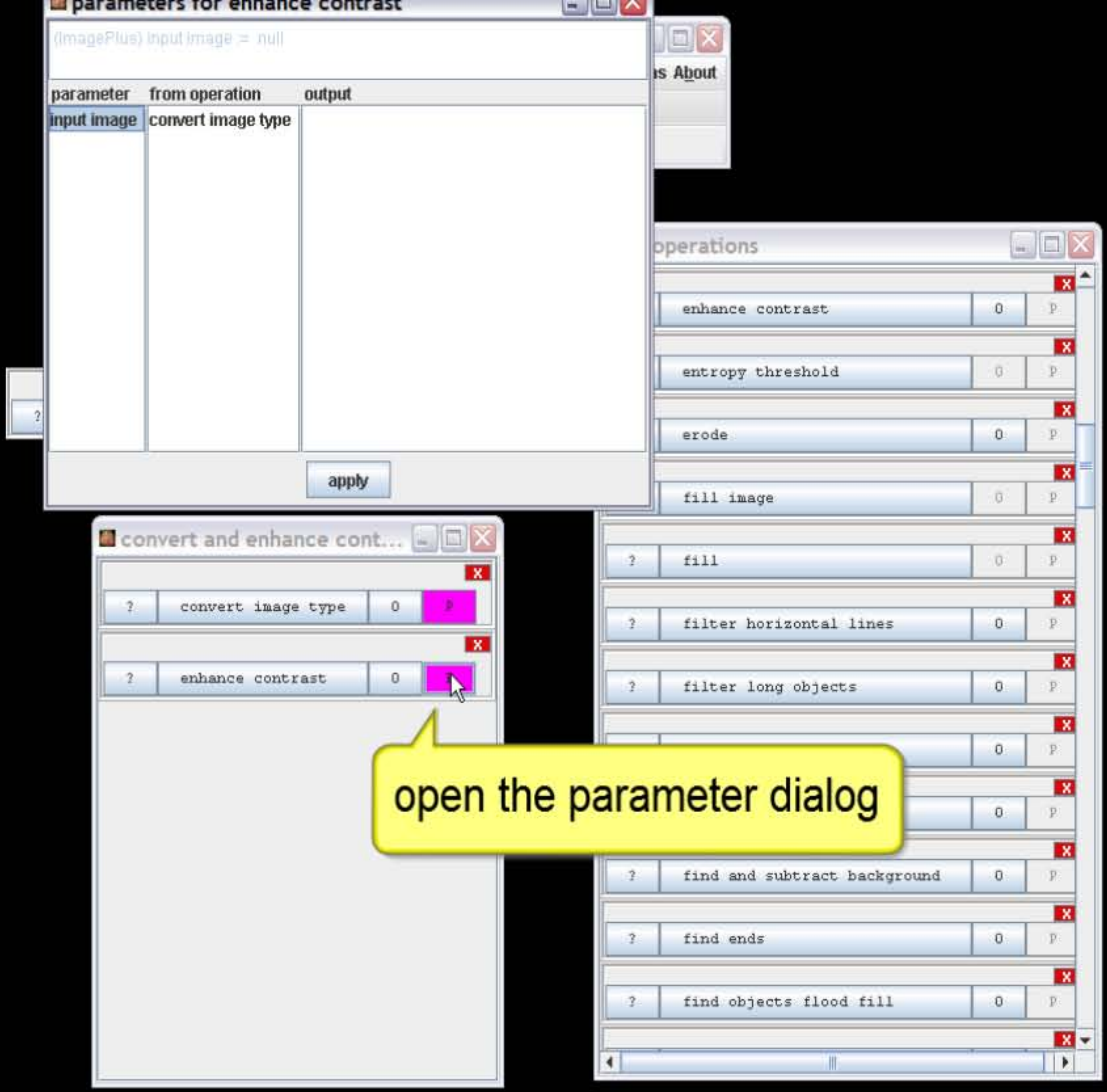
x=1224, y=468, value=21

Back Next



x=1224, y=468, value=21

Back Next



parameters for enhance contrast

(ImagePlus) input image = null

parameter	from operation	output
input image	convert image type	

apply

convert and enhance cont...

? convert image type 0 P

? enhance contrast 0 P

operations

enhance contrast 0 P

entropy threshold 0 P

erode 0 P

fill image 0 P

? fill 0 P

? filter horizontal lines 0 P

? filter long objects 0 P

0 P

0 P

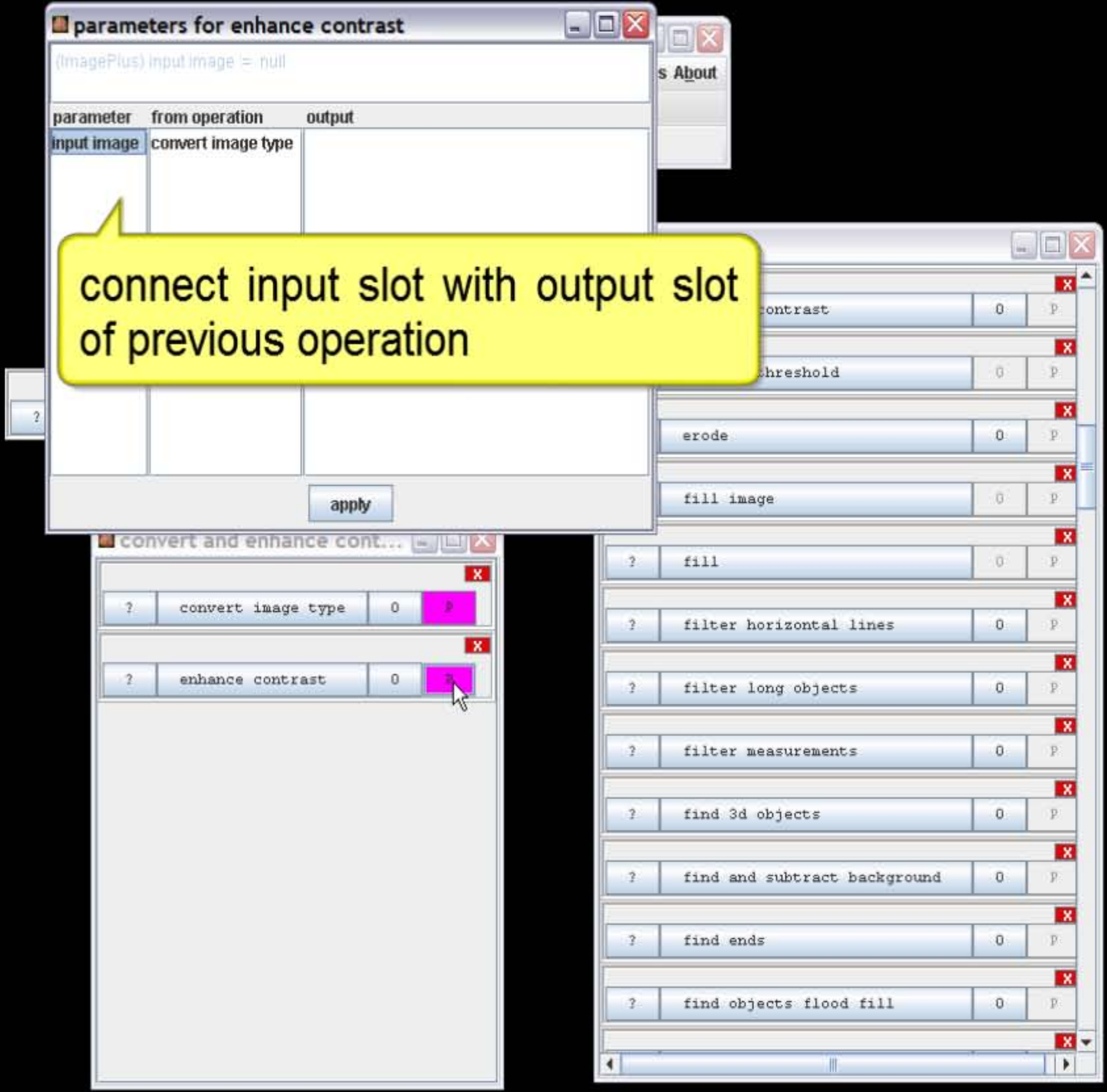
? find and subtract background 0 P

? find ends 0 P

? find objects flood fill 0 P

open the parameter dialog

Back Next



parameters for enhance contrast

(ImagePlus) input image = null

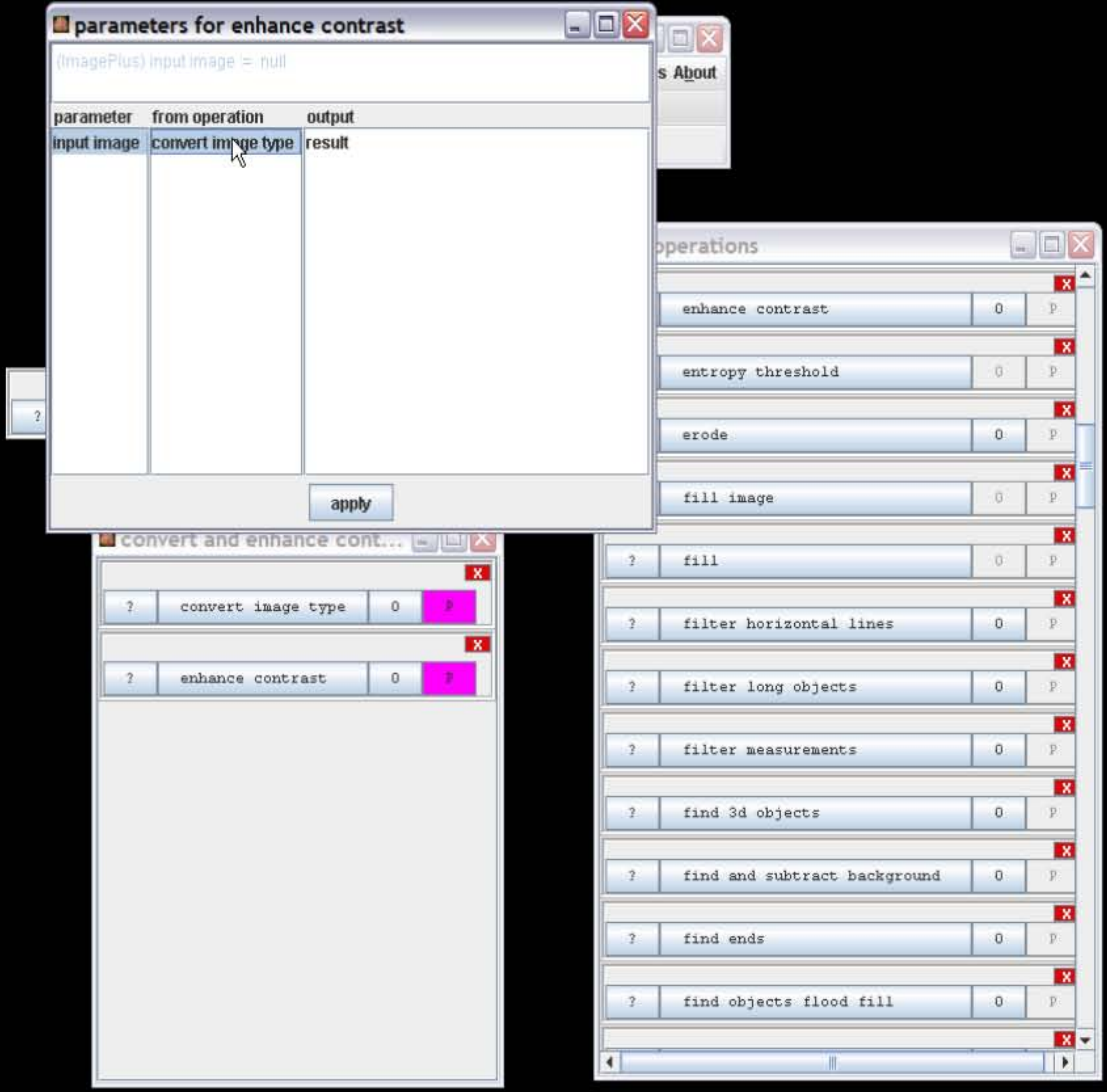
parameter	from operation	output
input image	convert image type	

apply

connect input slot with output slot of previous operation

s About
convert and enhance cont...
? convert image type 0 P
? enhance contrast 0 P
contrast 0 P
threshold 0 P
erode 0 P
fill image 0 P
? fill 0 P
? filter horizontal lines 0 P
? filter long objects 0 P
? filter measurements 0 P
? find 3d objects 0 P
? find and subtract background 0 P
? find ends 0 P
? find objects flood fill 0 P

Back Next

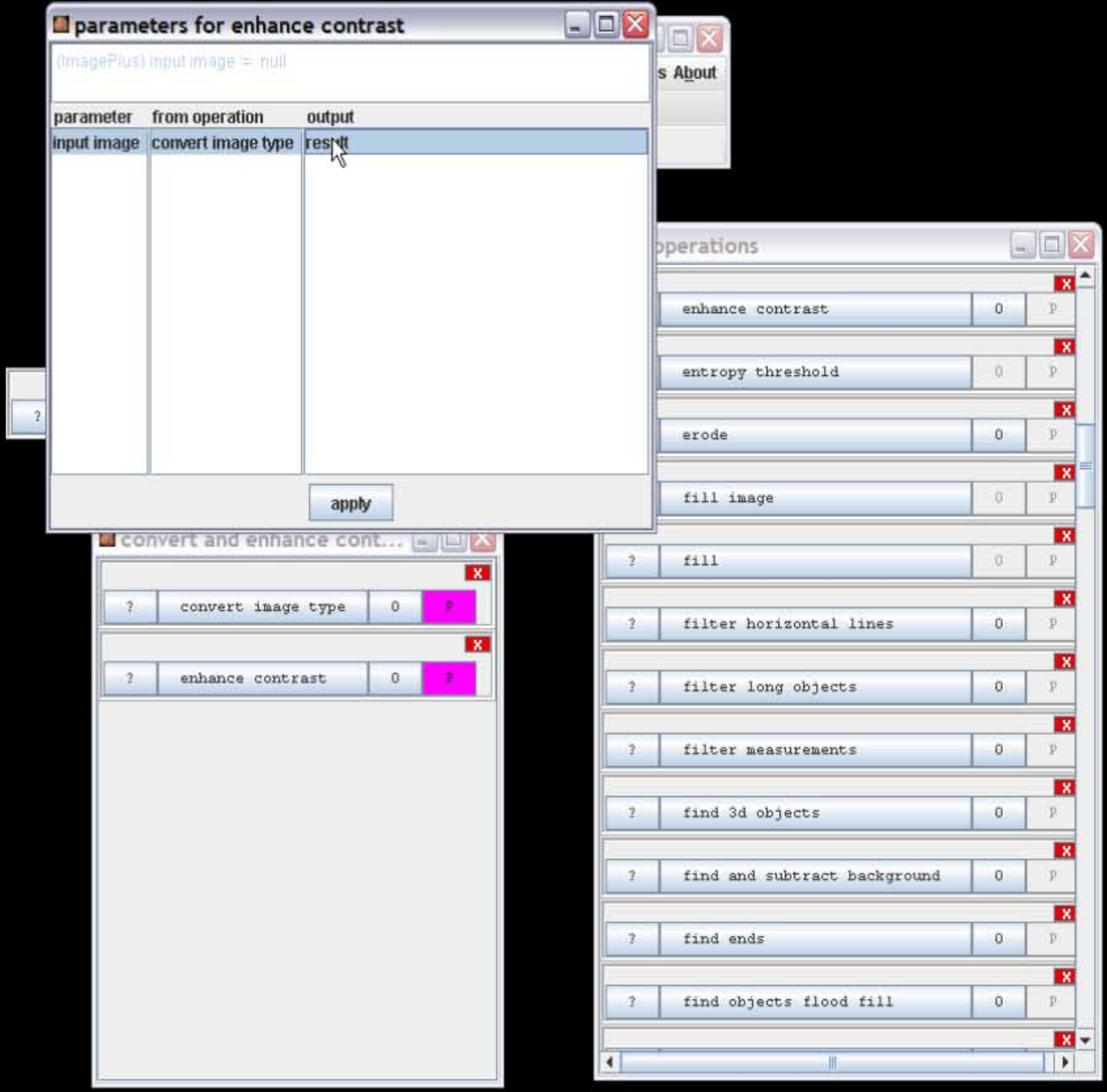


parameters for enhance contrast

(ImagePlus) input image = null

parameter	from operation	output
input image	convert image type	result

Back Next

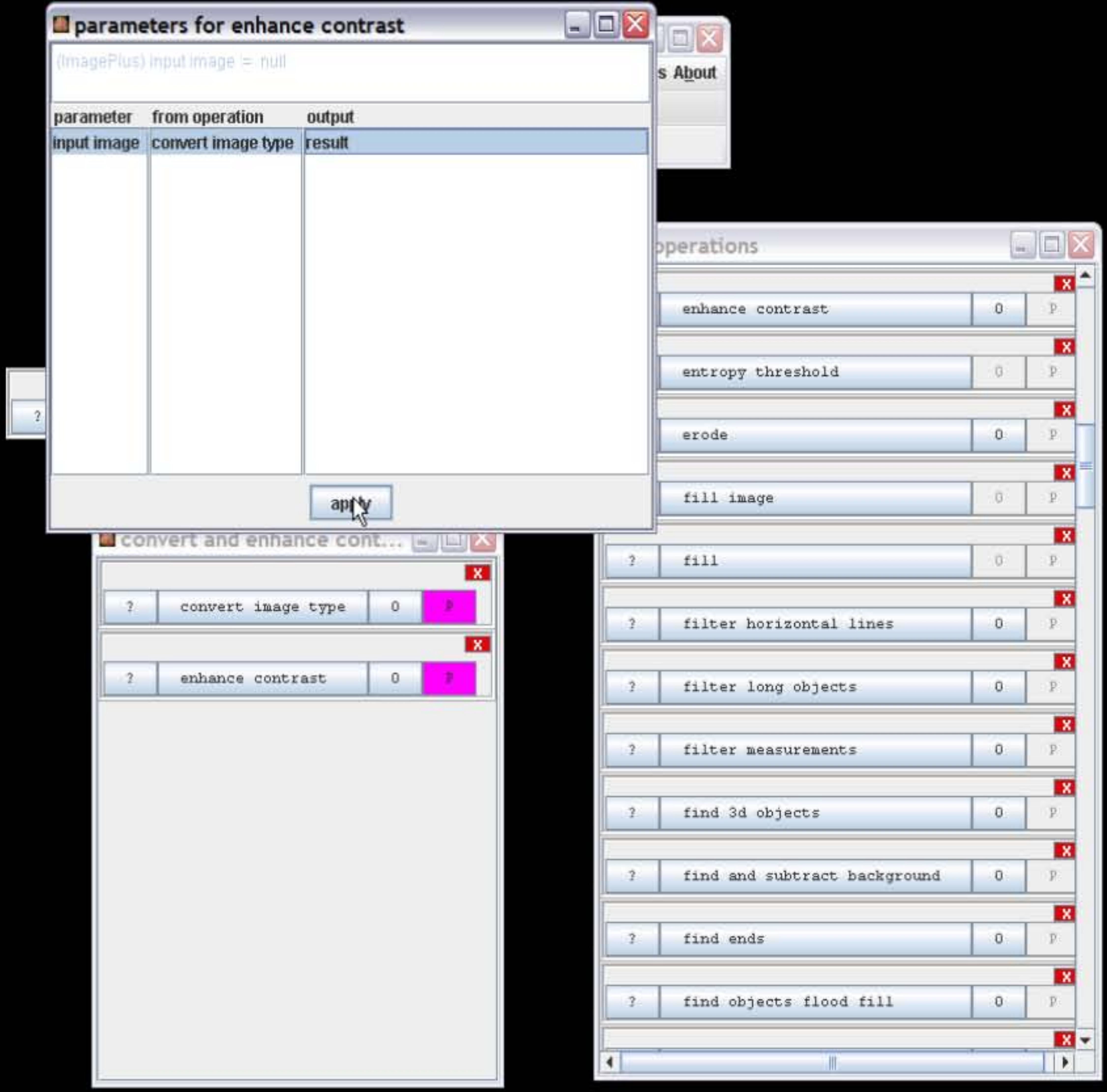


parameters for enhance contrast

(ImagePlus) input image := null

parameter	from operation	output
input image	convert image type	result

Back Next

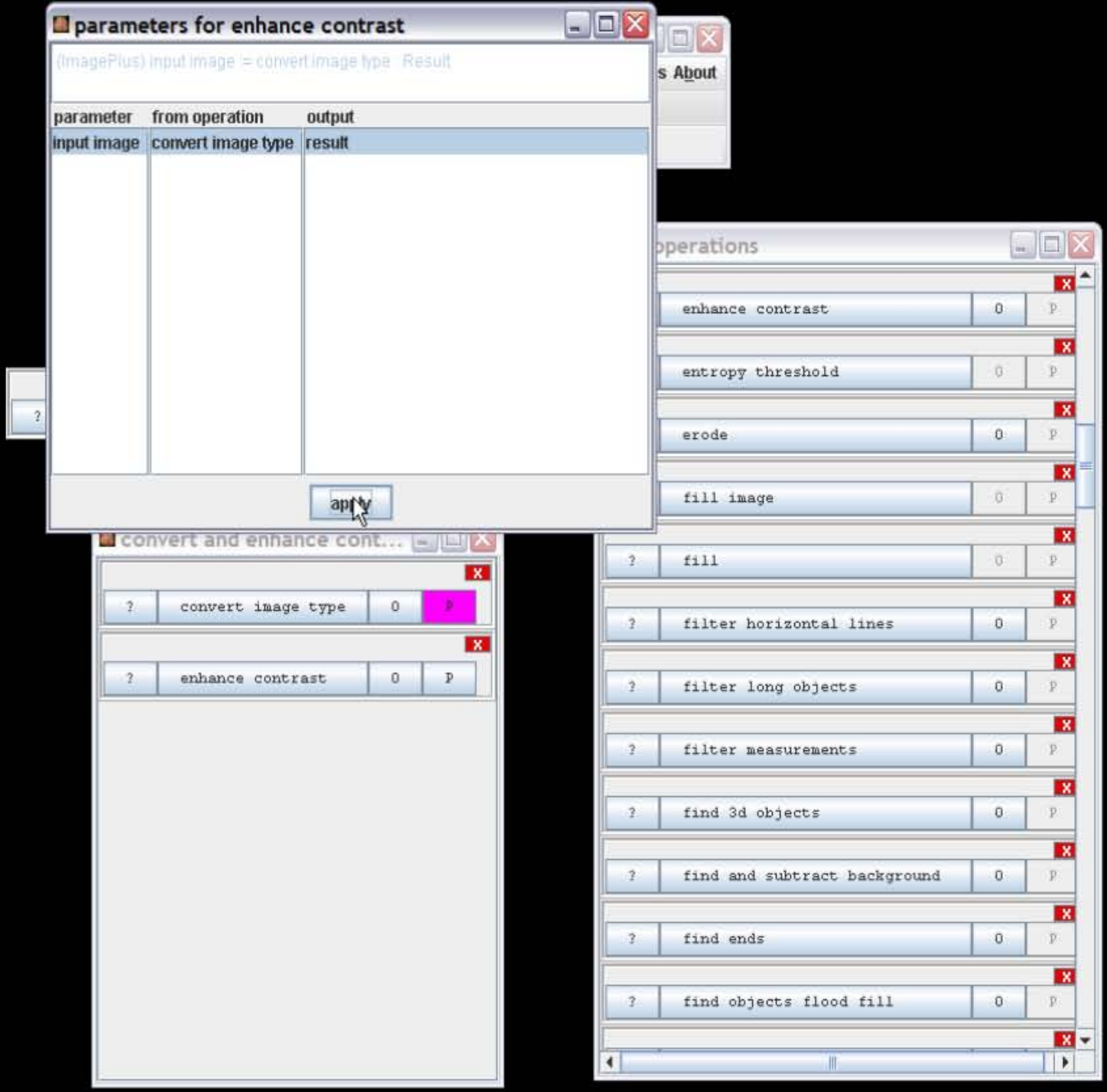


parameters for enhance contrast

(ImagePlus) input image != null

parameter	from operation	output
input image	convert image type	result

Back Next



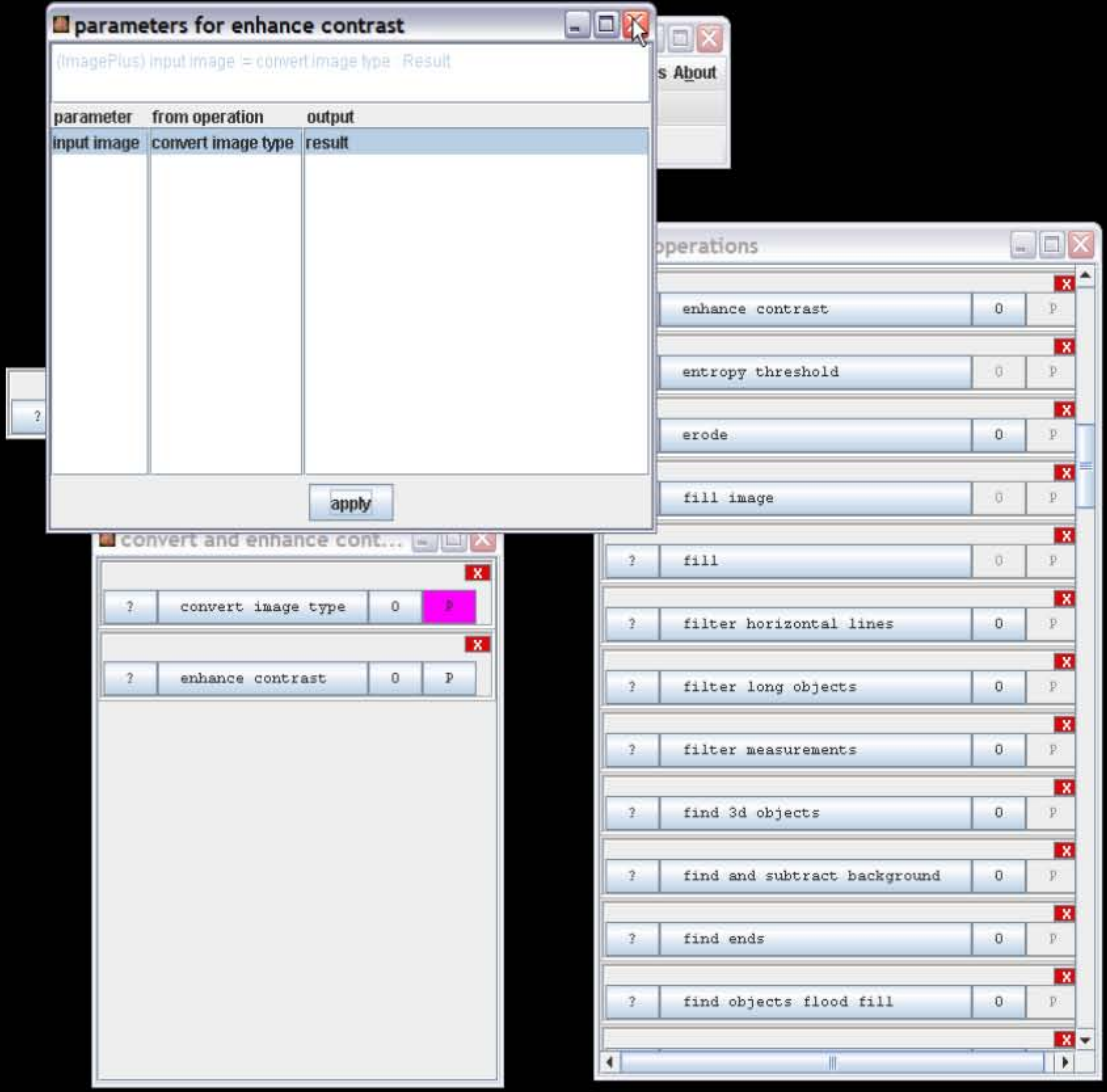
(ImagePlus) input image = convert image type . Result

parameter	from operation	output
input image	convert image type	result

?	convert image type	0	P
?	enhance contrast	0	P

	enhance contrast	0	P
	entropy threshold	0	P
	erode	0	P
	fill image	0	P
?	fill	0	P
?	filter horizontal lines	0	P
?	filter long objects	0	P
?	filter measurements	0	P
?	find 3d objects	0	P
?	find and subtract background	0	P
?	find ends	0	P
?	find objects flood fill	0	P

Back Next

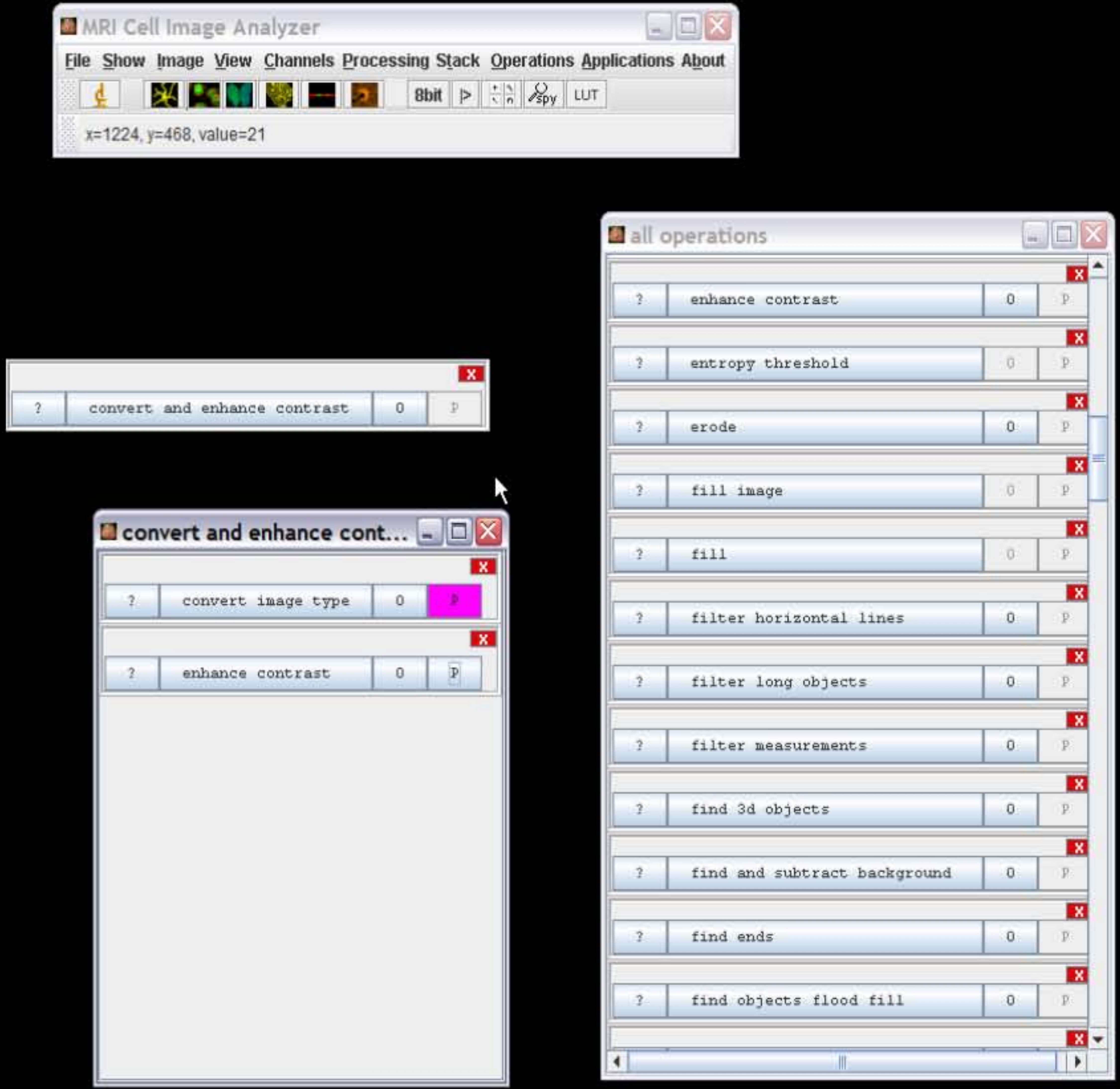


parameters for enhance contrast

(ImagePlus) input image := convert image type . Result

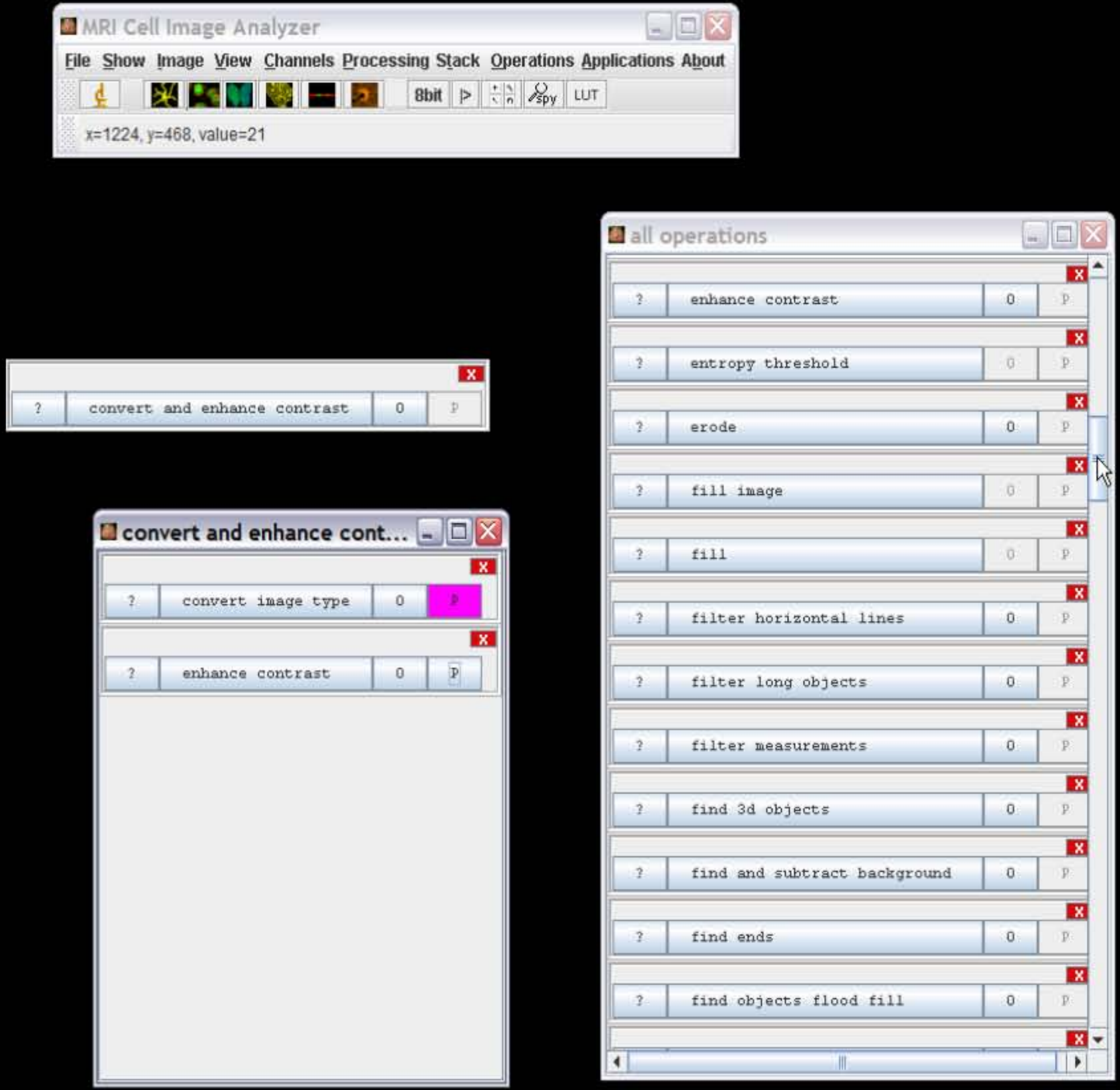
parameter	from operation	output
input image	convert image type	result

Back Next


MRI Cell Image Analyzer
File Show Image View Channels Processing Stack Operations Applications About
8bit
LUT
all operations
? enhance contrast 0 P
? entropy threshold 0 P
? erode 0 P
? fill image 0 P
? fill 0 P
? filter horizontal lines 0 P
? filter long objects 0 P
? filter measurements 0 P
? find 3d objects 0 P
? find and subtract background 0 P
? find ends 0 P
? find objects flood fill 0 P
? convert and enhance contrast 0 P
convert and enhance cont...
? convert image type 0 P
? enhance contrast 0 P

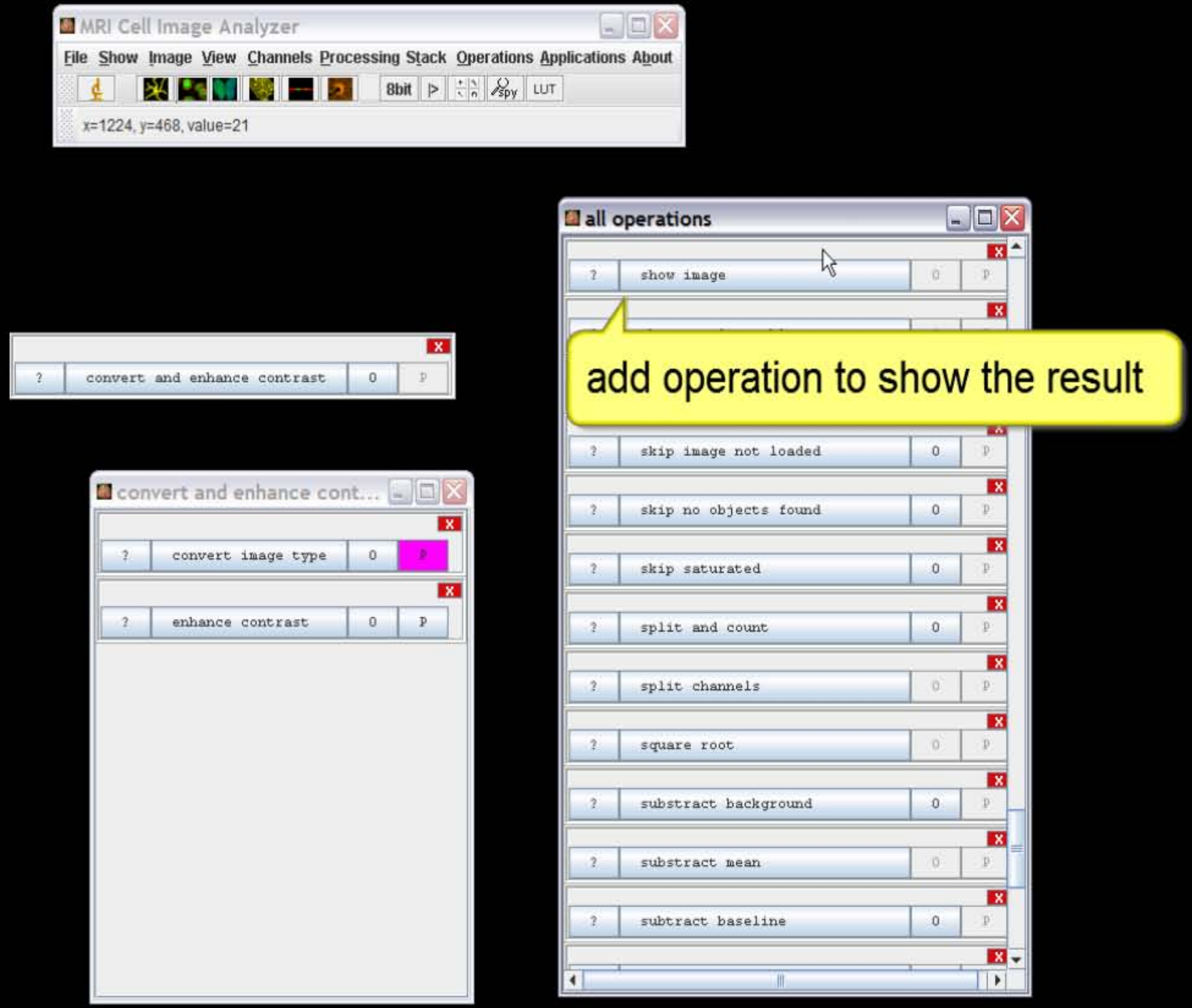
x=1224, y=468, value=21

Back Next


MRI Cell Image Analyzer
File Show Image View Channels Processing Stack Operations Applications About
8bit
LUT
all operations
? enhance contrast 0 P
? entropy threshold 0 P
? erode 0 P
? fill image 0 P
? fill 0 P
? filter horizontal lines 0 P
? filter long objects 0 P
? filter measurements 0 P
? find 3d objects 0 P
? find and subtract background 0 P
? find ends 0 P
? find objects flood fill 0 P
? convert and enhance contrast 0 P
convert and enhance cont...
? convert image type 0 P
? enhance contrast 0 P

x=1224, y=468, value=21

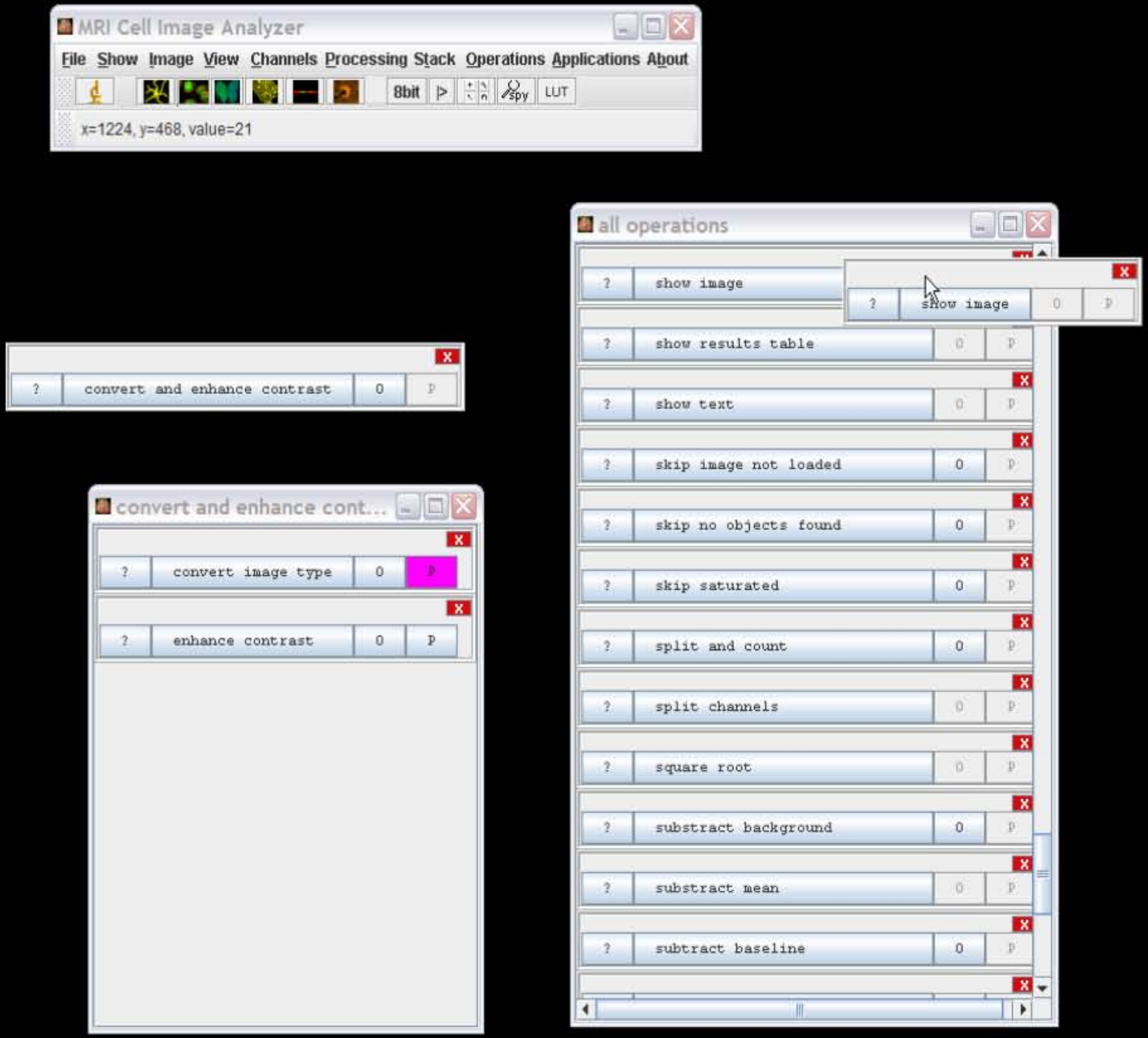
Back Next



x=1224, y=468, value=21

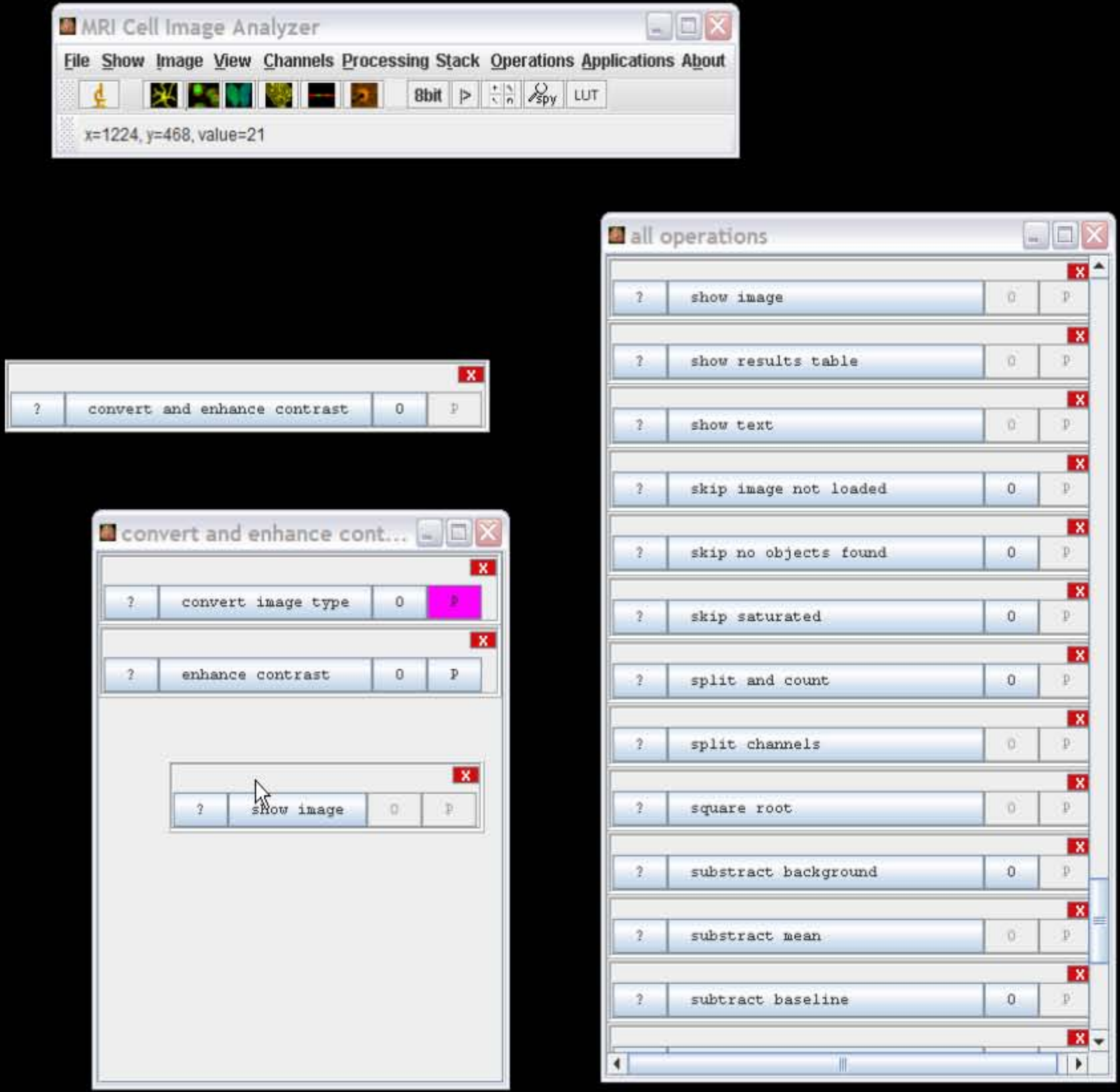
add operation to show the result

Back Next



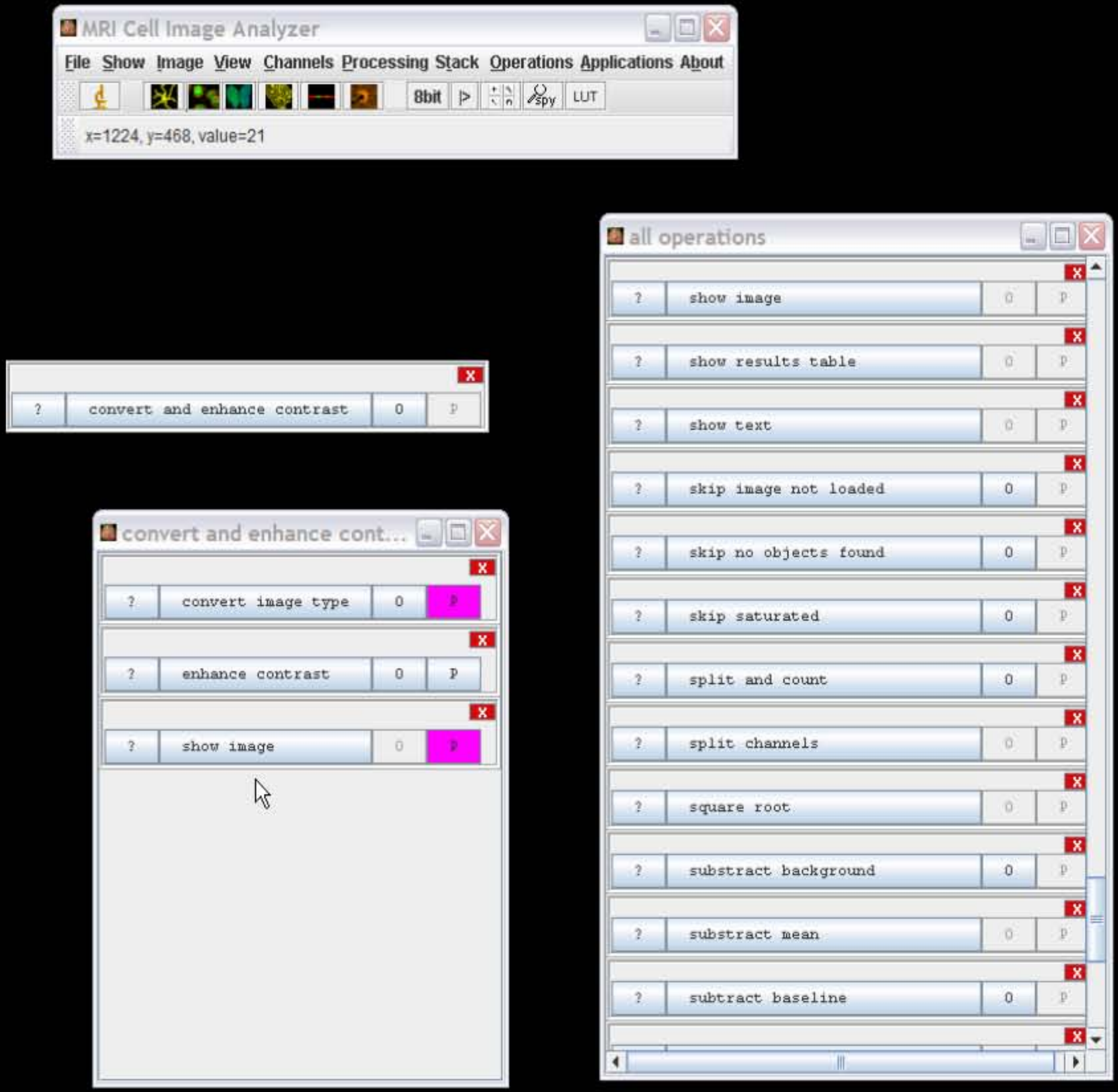
x=1224, y=468, value=21

Back Next



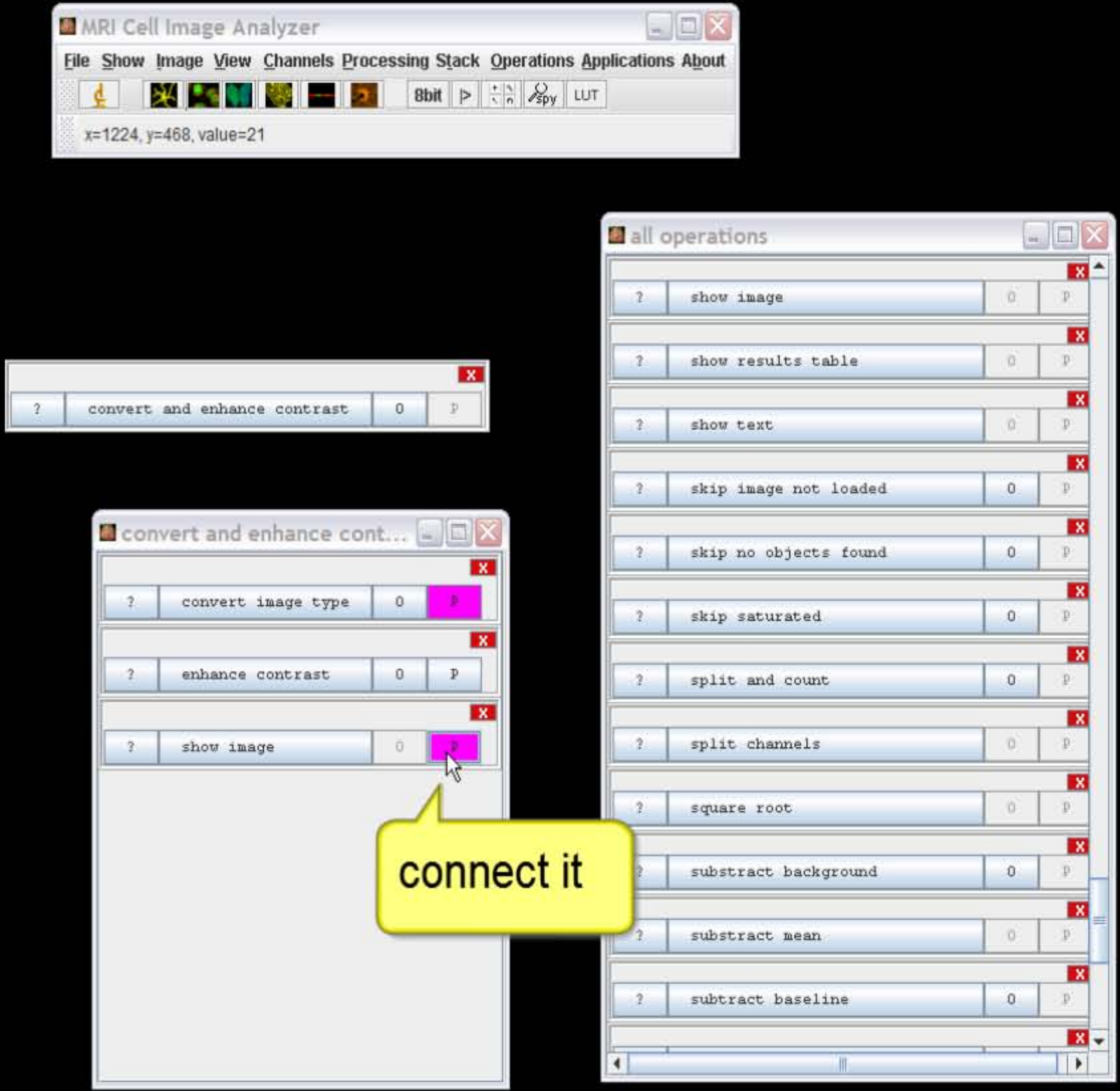
x=1224, y=468, value=21

Back Next



x=1224, y=468, value=21

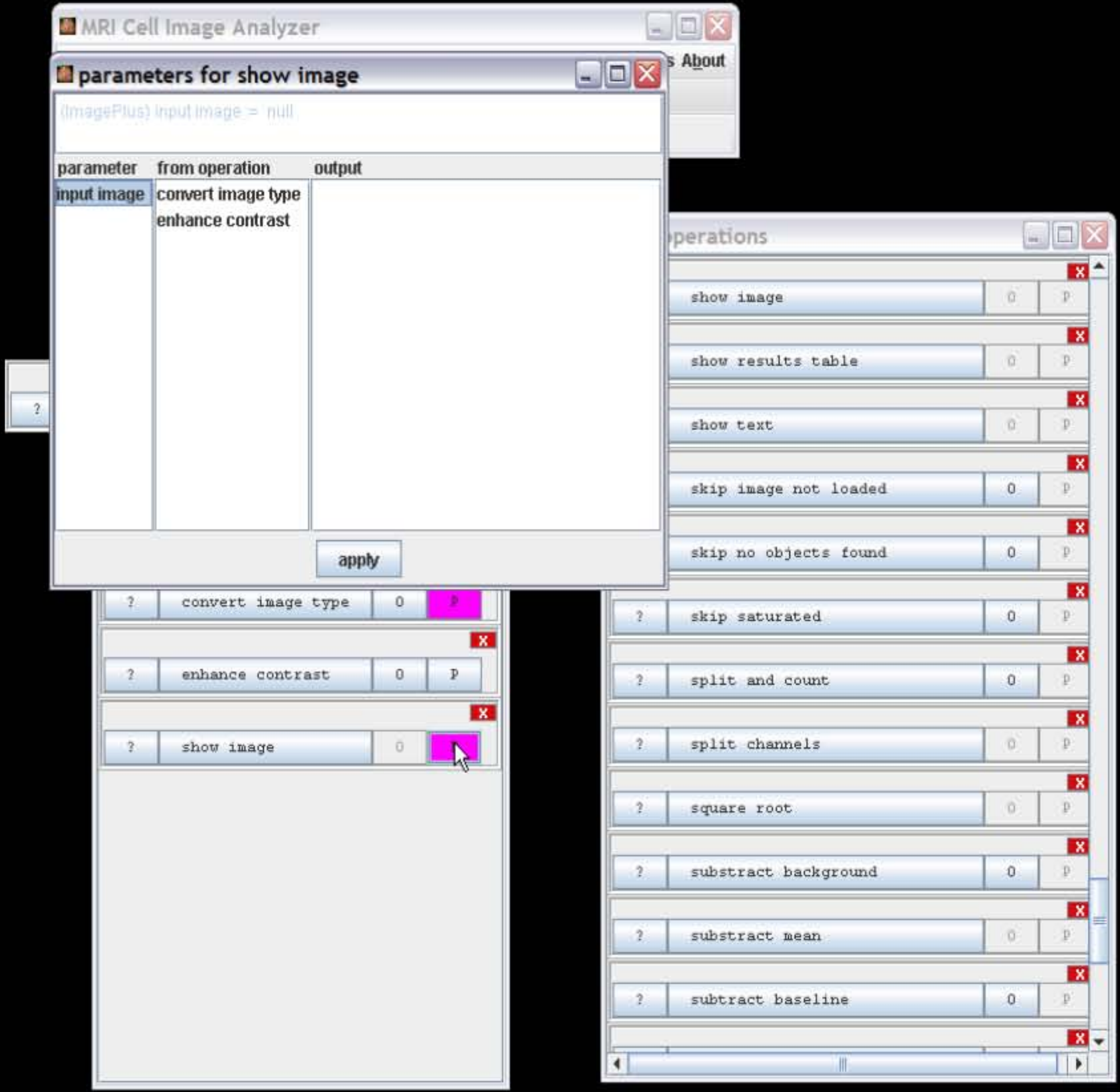
Back Next



x=1224, y=468, value=21

connect it

Back Next



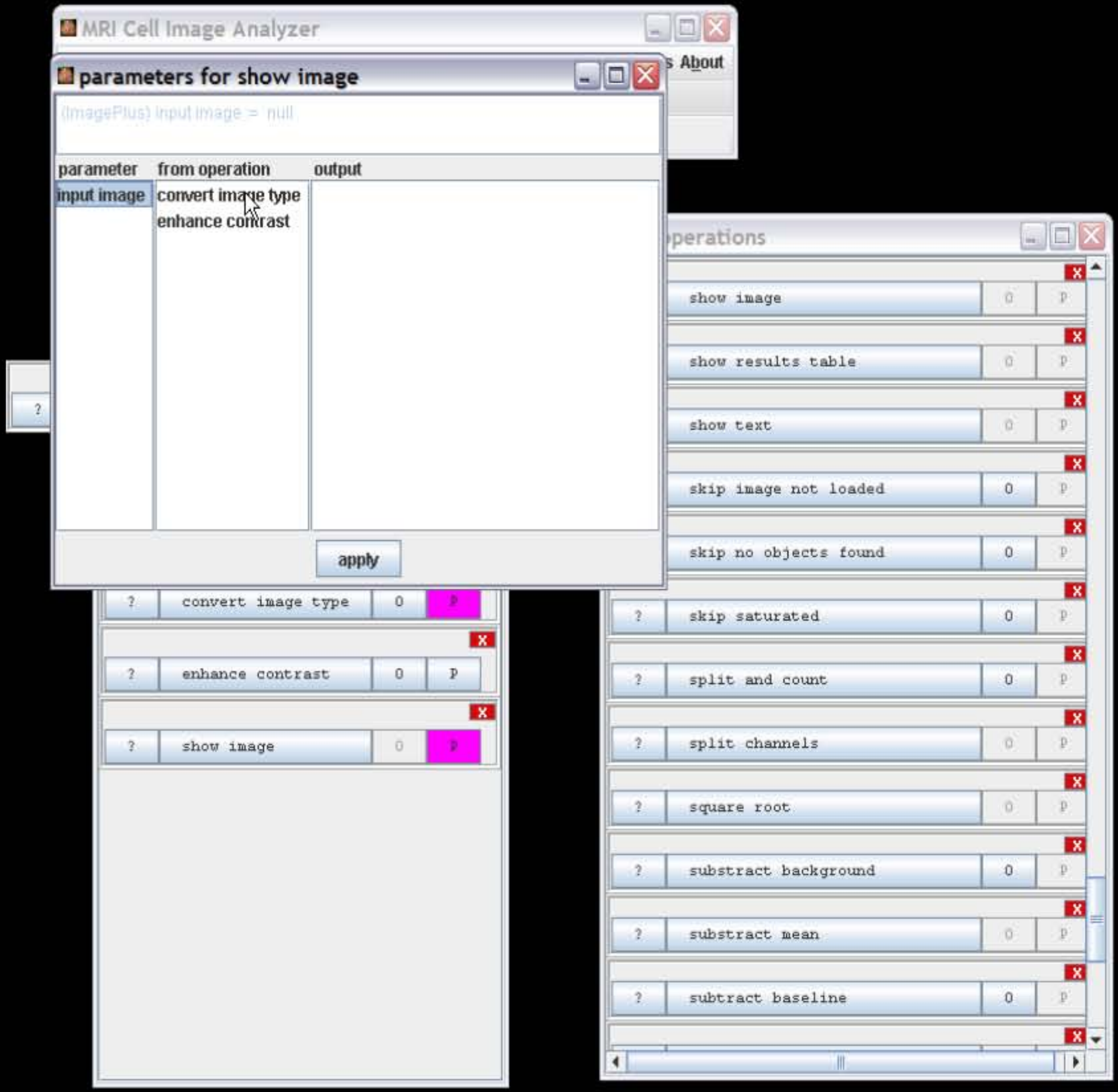
MRI Cell Image Analyzer

parameters for show image

(ImagePlus) input image = null

parameter	from operation	output
input image	convert image type enhance contrast	

Back Next



parameters for show image

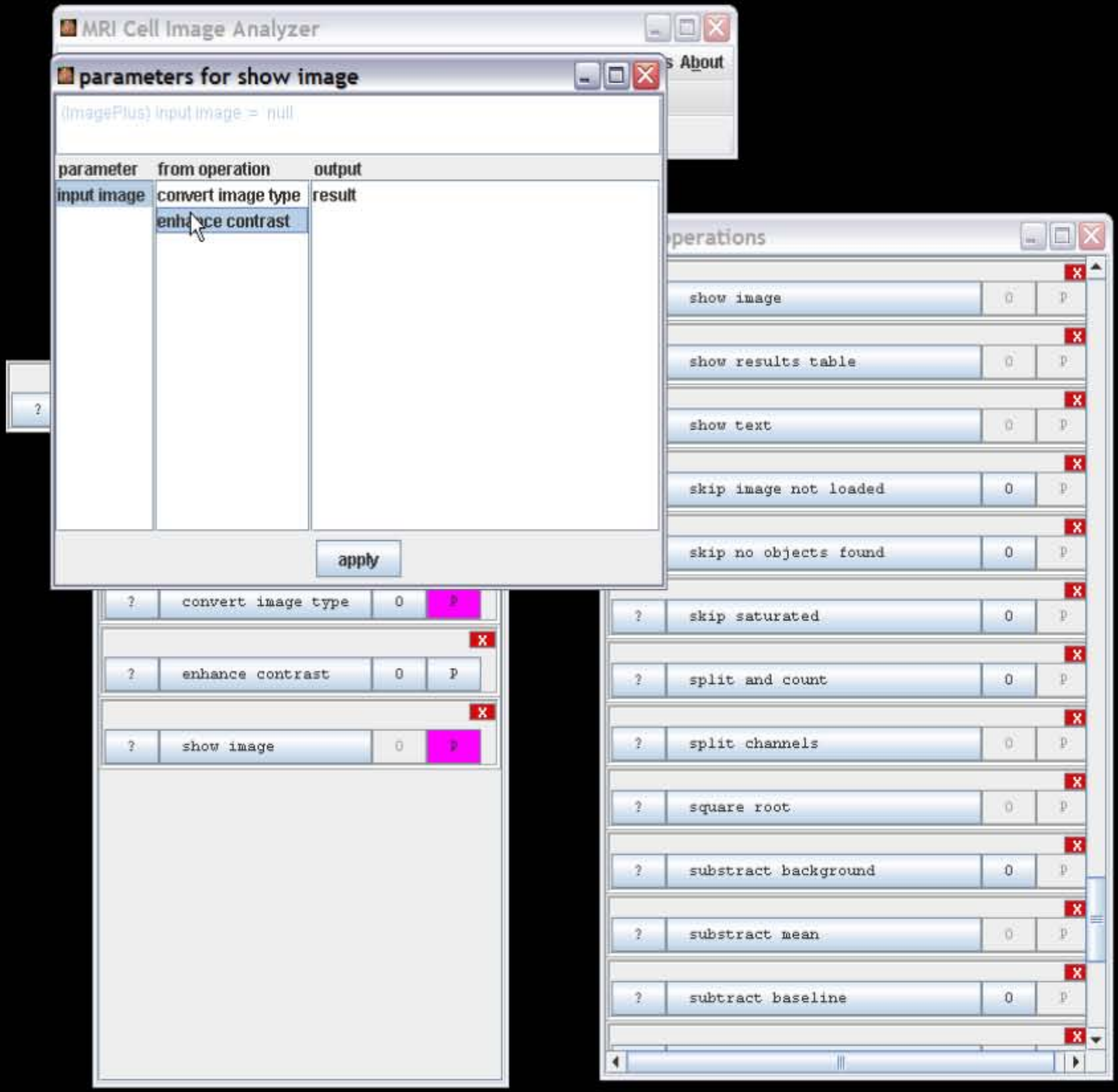
(ImagePlus) input image = null

parameter	from operation	output
input image	convert image type enhance contrast	

```
?  convert image type   0  P
?  enhance contrast     0  P
?  show image           0  P
```

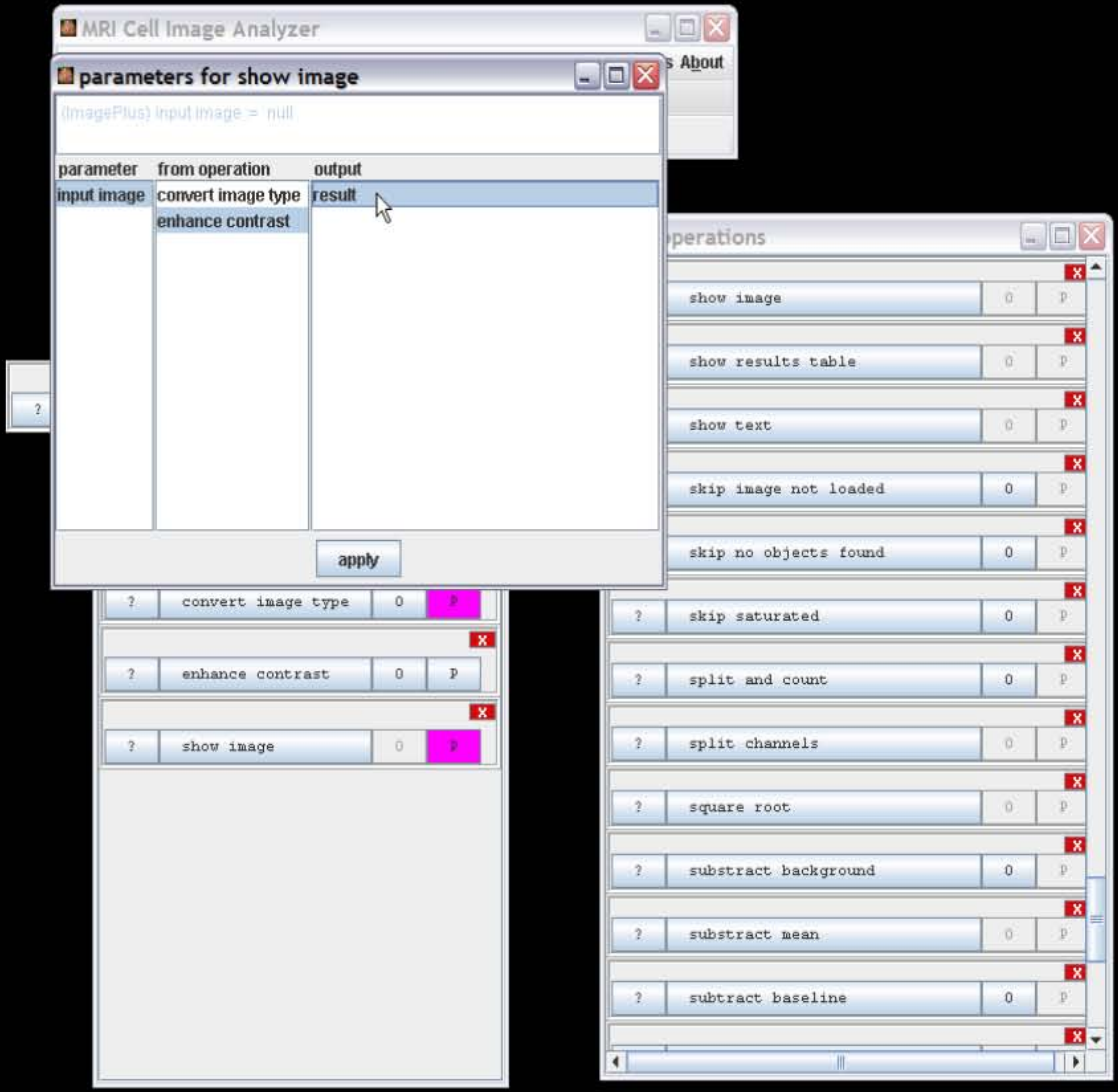
```
show image              0  P
show results table      0  P
show text               0  P
skip image not loaded   0  P
skip no objects found   0  P
?  skip saturated       0  P
?  split and count      0  P
?  split channels       0  P
?  square root          0  P
?  substract background 0  P
?  substract mean       0  P
?  subtract baseline    0  P
```

Back Next



parameter	from operation	output
input image	convert image type	result
	enhance contrast	

Back Next

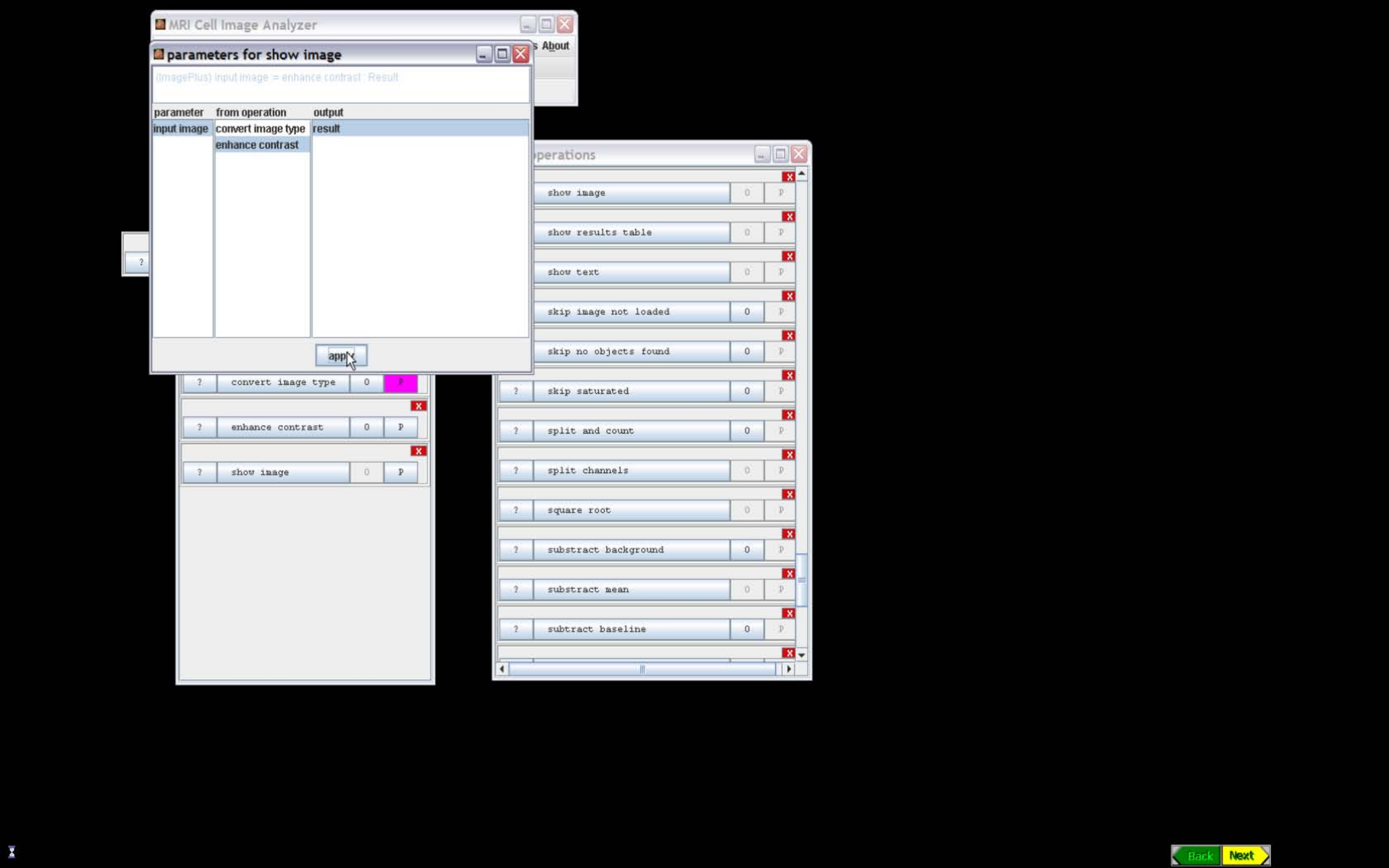


MRI Cell Image Analyzer

parameters for show image

(ImagePlus) input image = null

parameter	from operation	output
input image	convert image type	result
	enhance contrast	

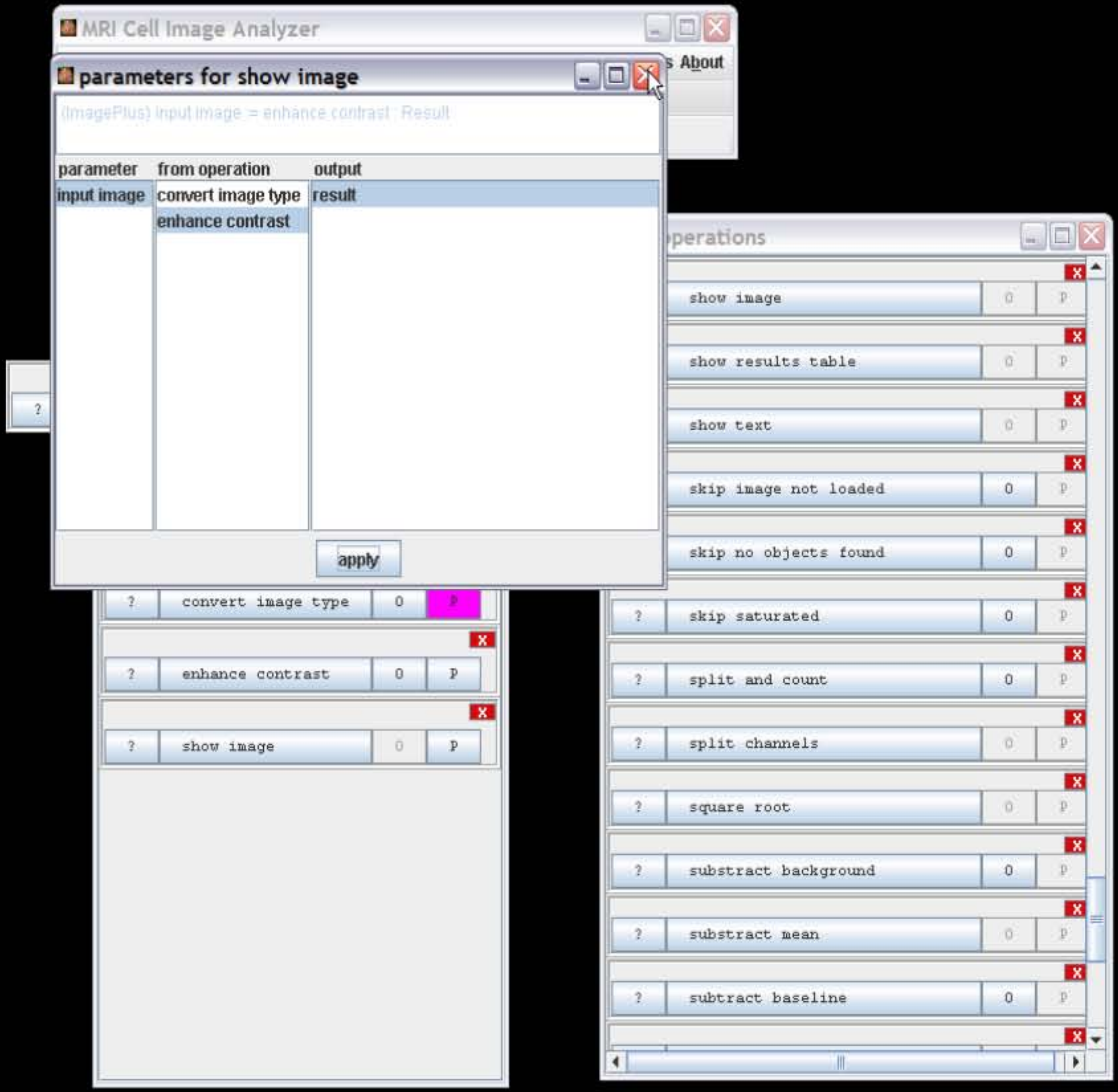


MRI Cell Image Analyzer

parameters for show image

(ImagePlus) input image = enhance contrast : Result

parameter	from operation	output
input image	convert image type	result
	enhance contrast	



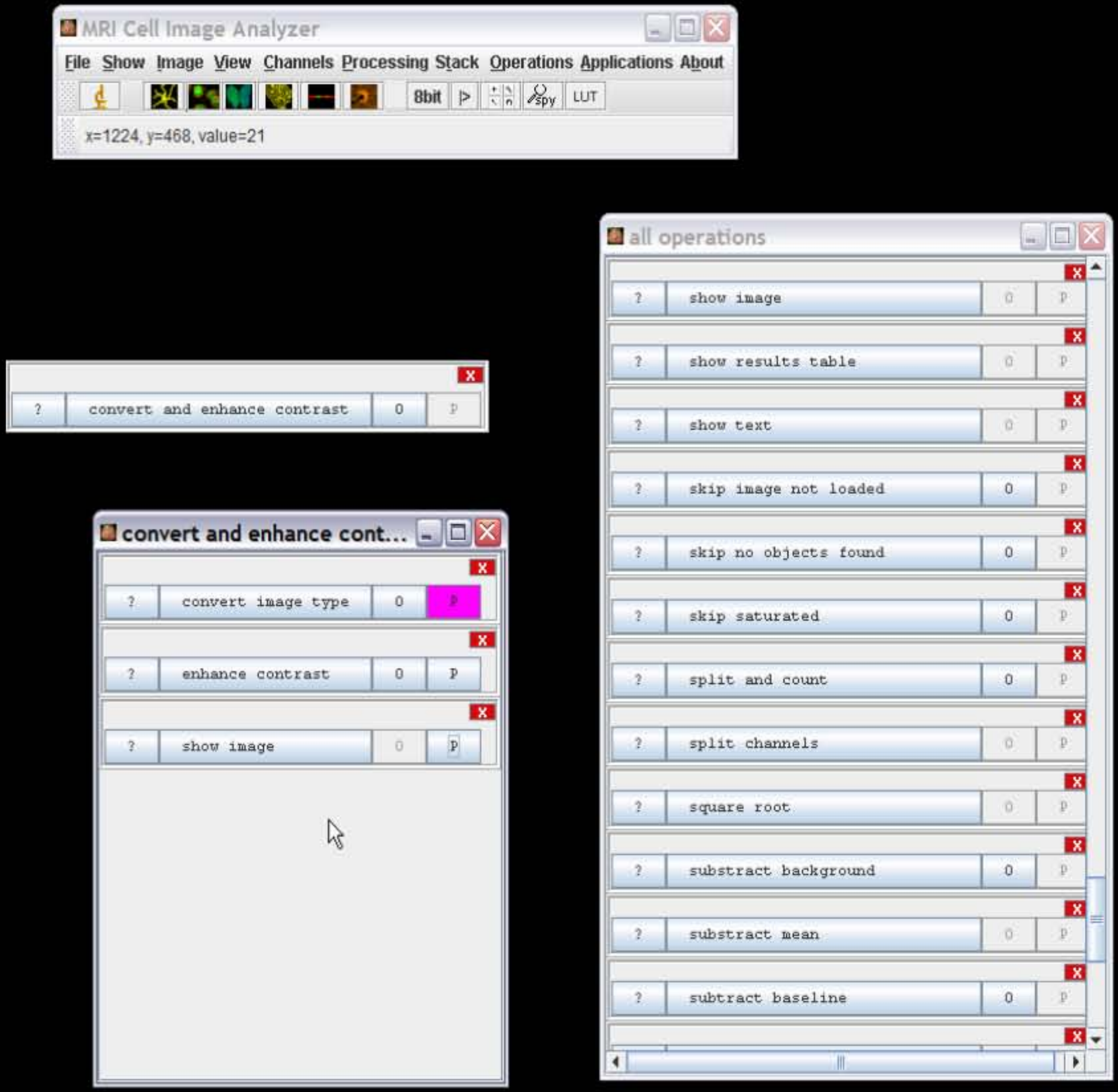
(ImagePlus) input image = enhance contrast : Result

parameter	from operation	output
input image	convert image type	result
	enhance contrast	

?	convert image type	0	P
?	enhance contrast	0	P
?	show image	0	P

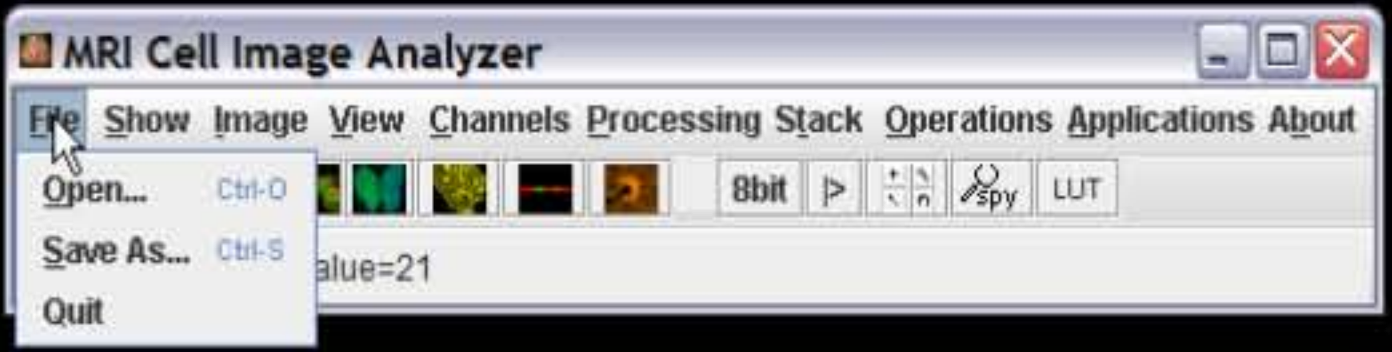
	show image	0	P
	show results table	0	P
	show text	0	P
	skip image not loaded	0	P
	skip no objects found	0	P
?	skip saturated	0	P
?	split and count	0	P
?	split channels	0	P
?	square root	0	P
?	substract background	0	P
?	substract mean	0	P
?	subtract baseline	0	P

Back Next

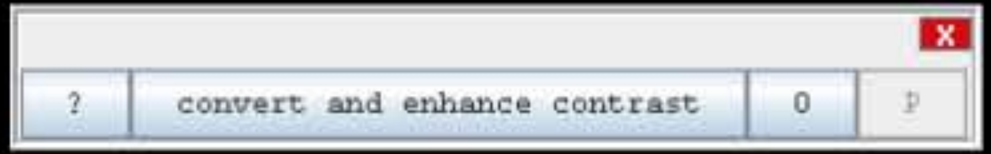


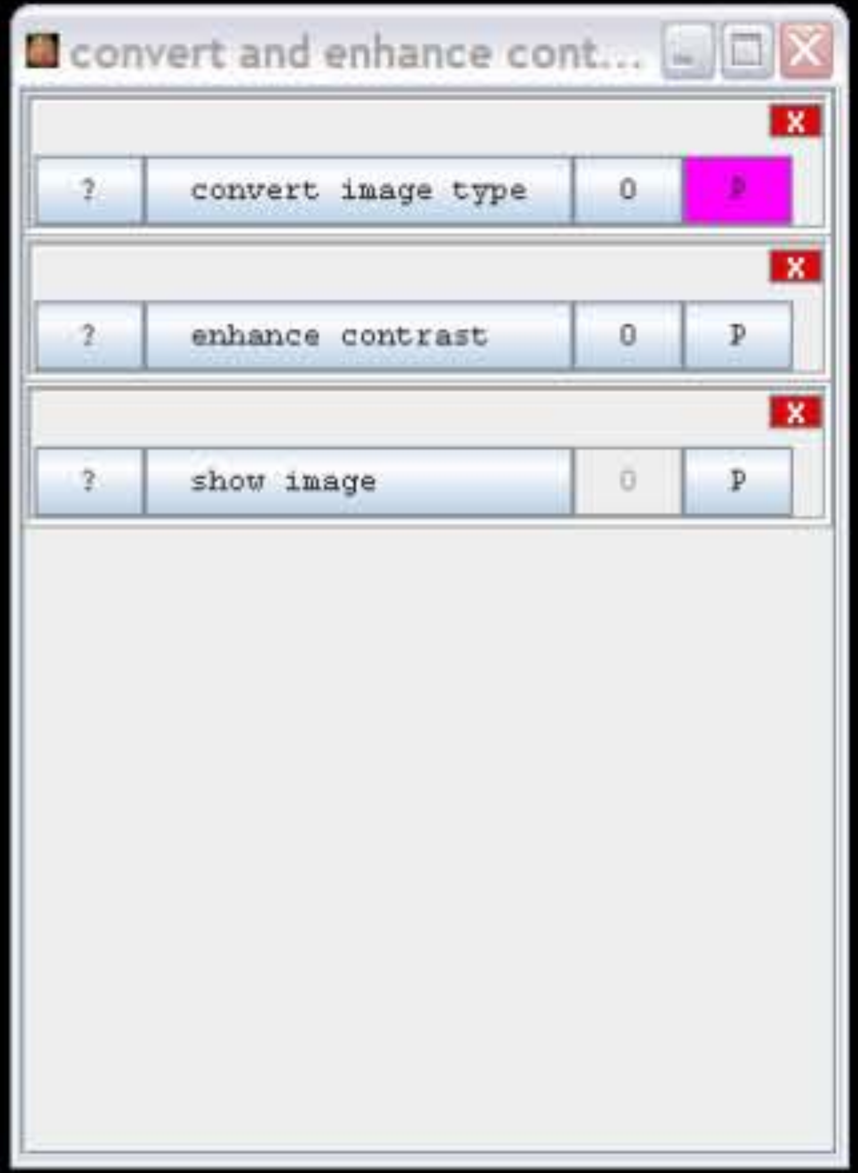
x=1224, y=468, value=21

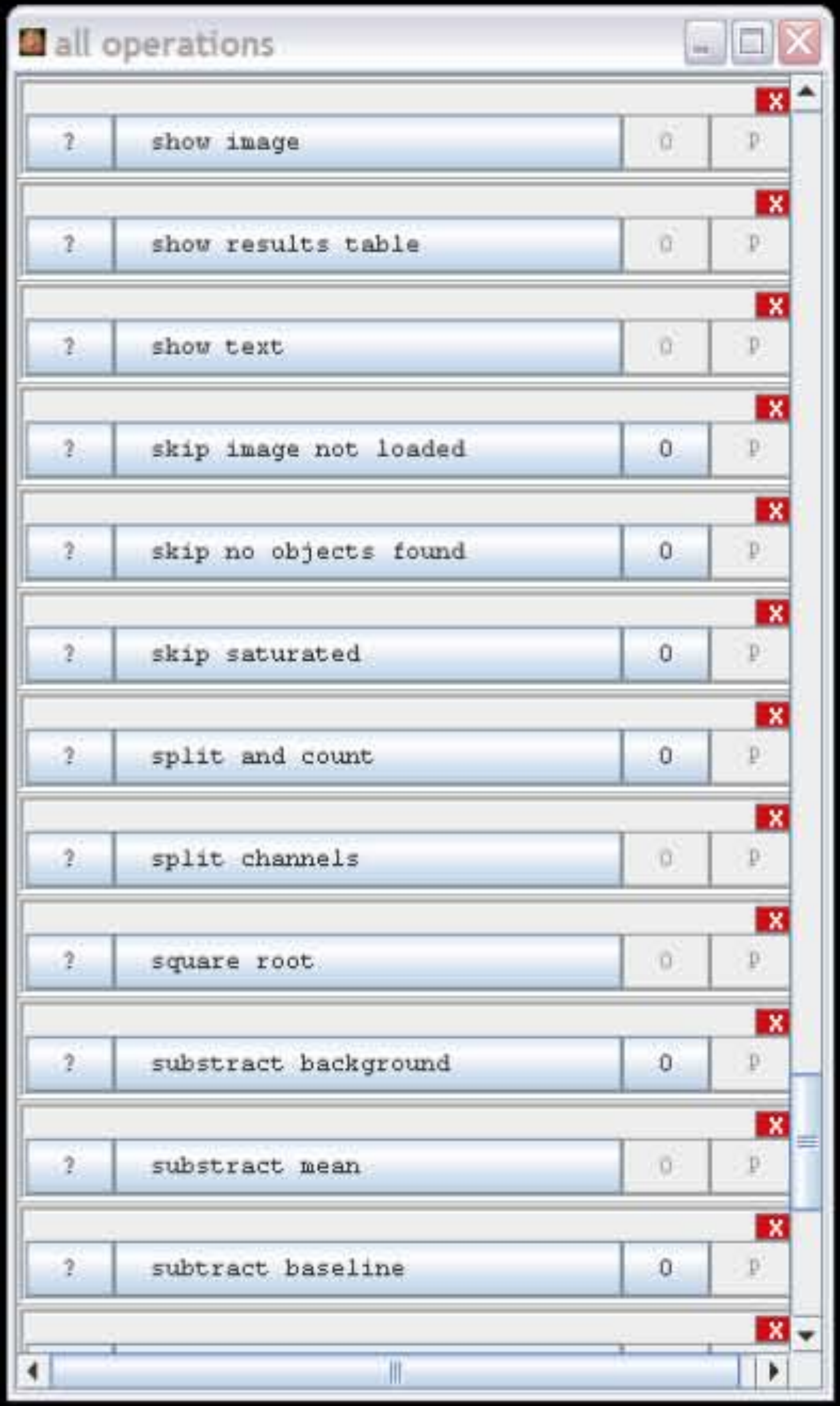
Back Next


MRI Cell Image Analyzer
File Show Image View Channels Processing Stack Operations Applications About
Open... Ctrl-O
Save As... Ctrl-S
Quit
8bit
LUT
alue=21

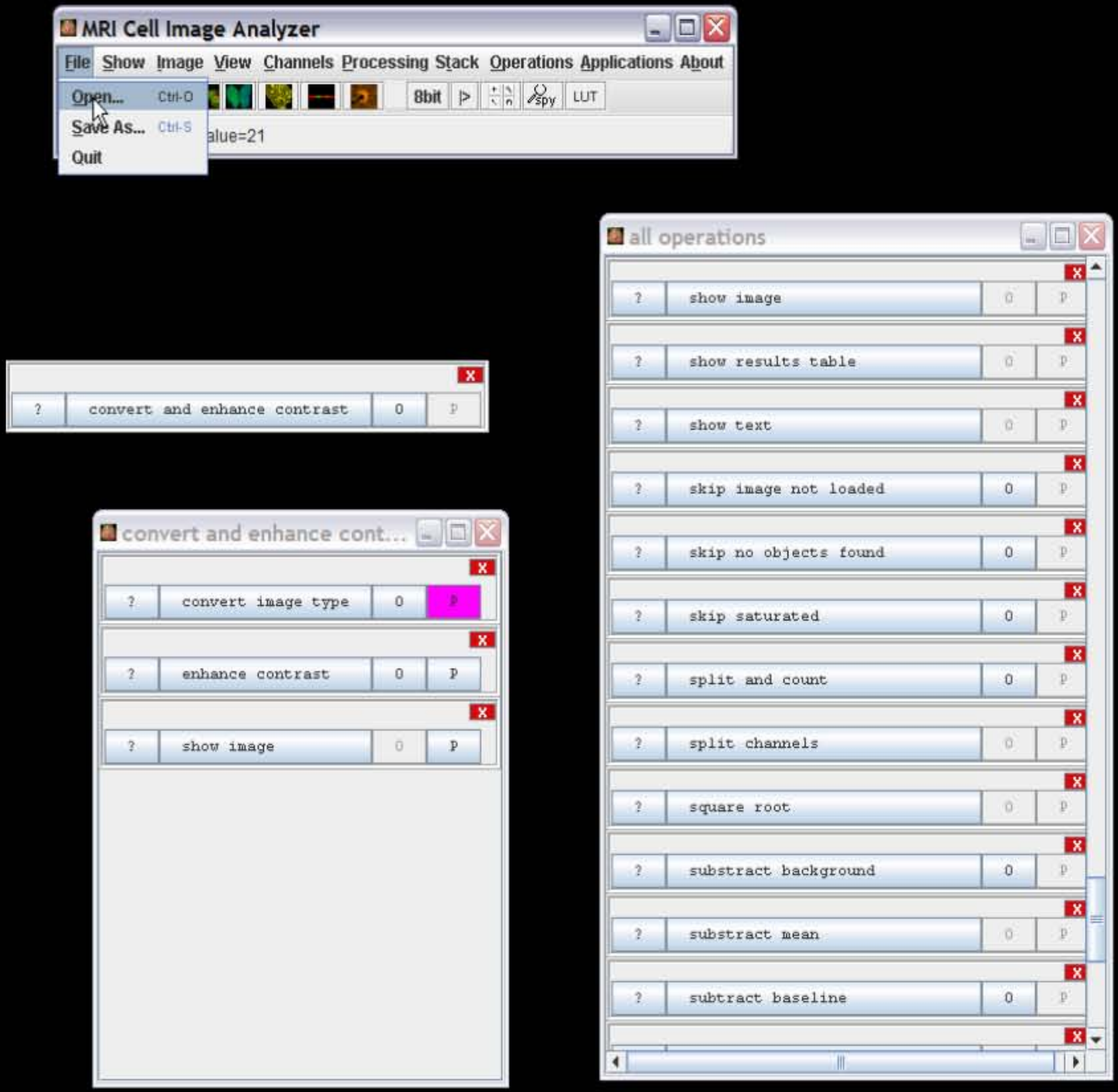
open an image



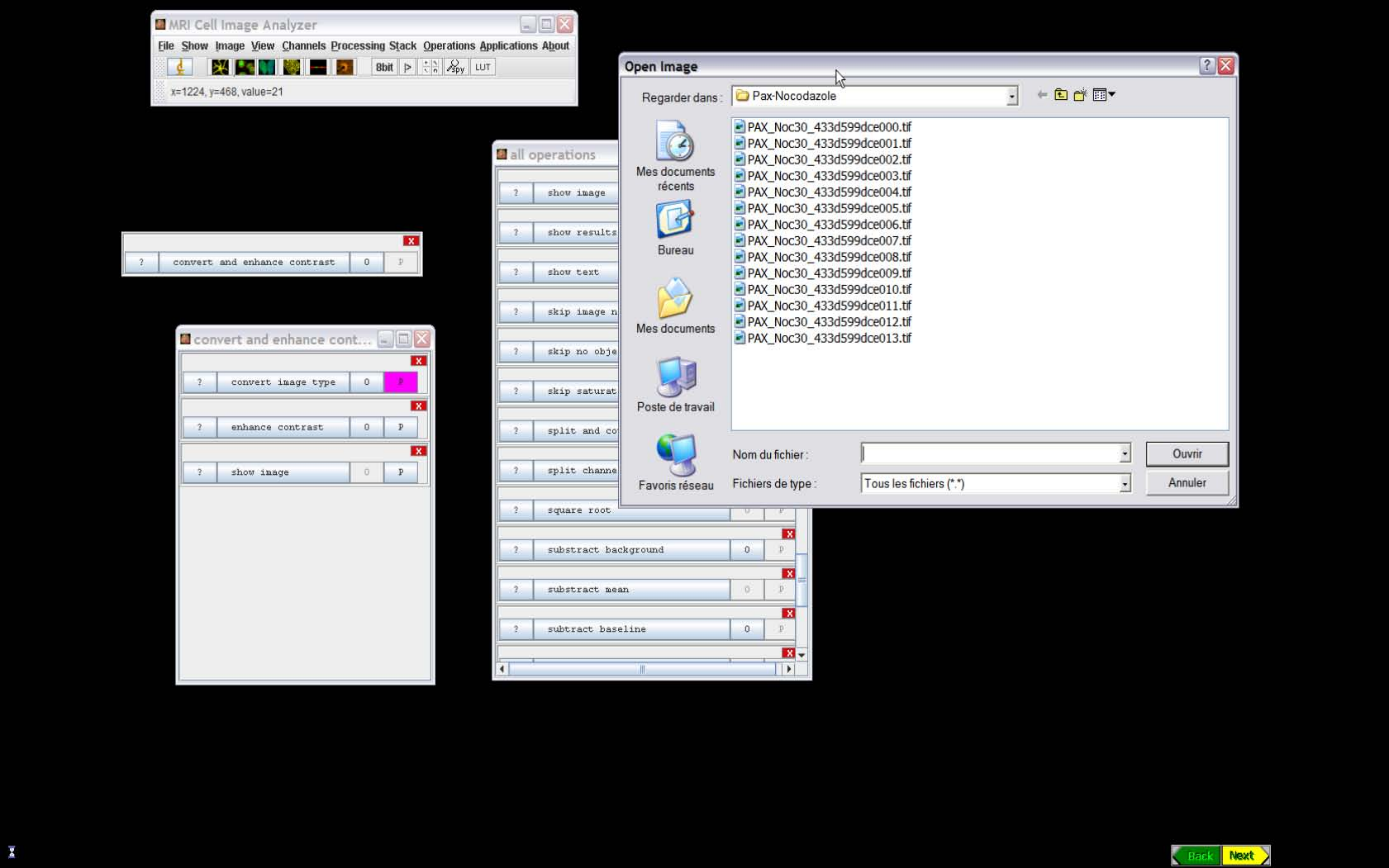




Back Next


MRI Cell Image Analyzer
File Show Image View Channels Processing Stack Operations Applications About
Open... Ctrl-O
Save As... Ctrl-S
Quit
8bit
LUT
spy
alue=21
all operations
? show image 0 P
? show results table 0 P
? show text 0 P
? skip image not loaded 0 P
? skip no objects found 0 P
? skip saturated 0 P
? split and count 0 P
? split channels 0 P
? square root 0 P
? substract background 0 P
? substract mean 0 P
? subtract baseline 0 P
? convert and enhance contrast 0 P
convert and enhance cont...
? convert image type 0 P
? enhance contrast 0 P
? show image 0 P

Back Next


MRI Cell Image Analyzer
File Show Image View Channels Processing Stack Operations Applications About
8bit
LUT
all operations
convert and enhance cont...
Back
Next

x=1224, y=468, value=21

?	convert and enhance contrast	0	P

?	convert image type	0	P
?	enhance contrast	0	P
?	show image	0	P

?	show image
?	show results
?	show text
?	skip image n
?	skip no obje
?	skip saturat
?	split and co
?	split channe

?	square root	0	P
?	substract background	0	P
?	substract mean	0	P
?	subtract baseline	0	P

Open Image

Regarder dans : Pax-Nocodazole

Mes documents récents
Bureau
Mes documents
Poste de travail
Favoris réseau

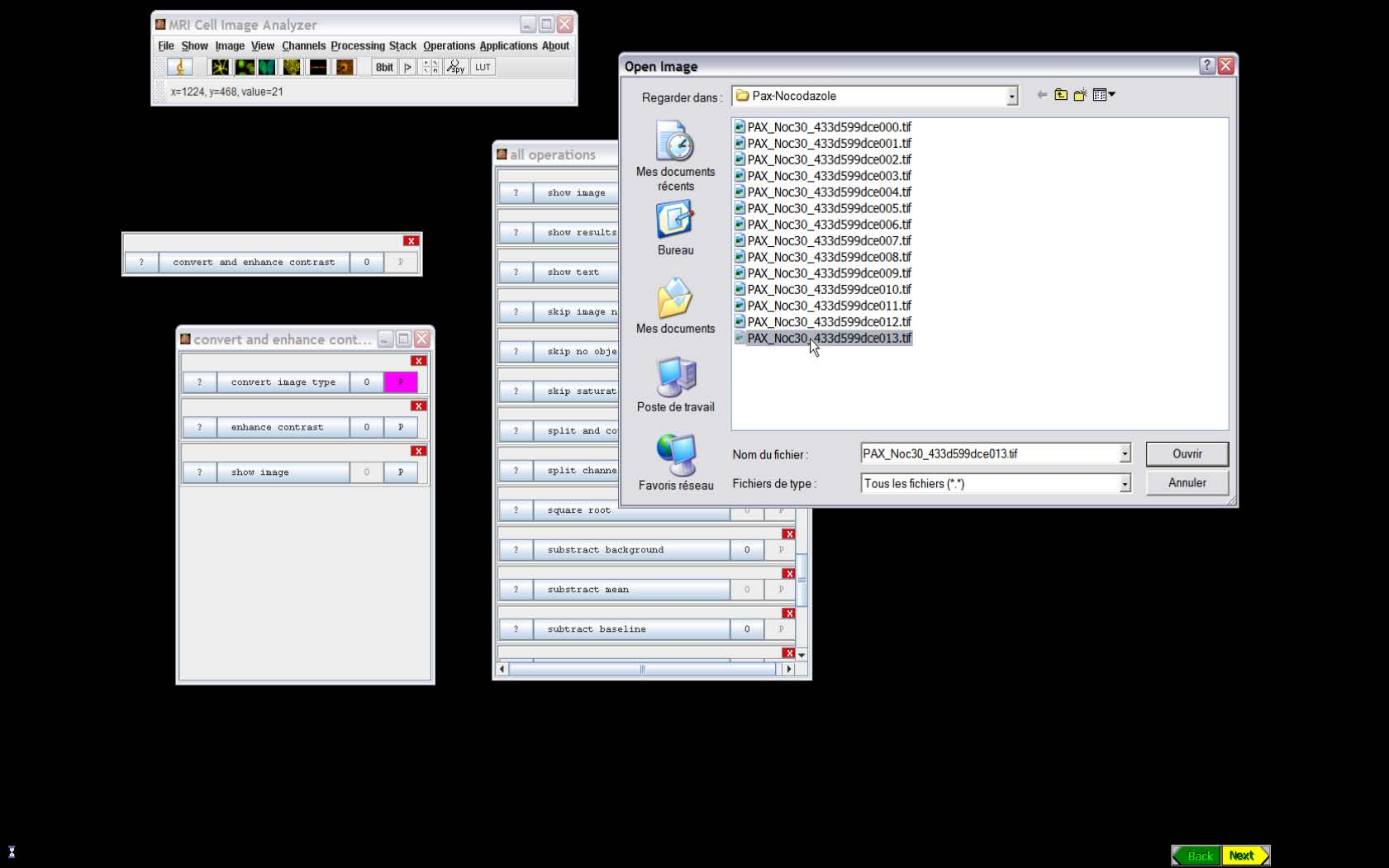
PAX_Noc30_433d599dce000.tif
PAX_Noc30_433d599dce001.tif
PAX_Noc30_433d599dce002.tif
PAX_Noc30_433d599dce003.tif
PAX_Noc30_433d599dce004.tif
PAX_Noc30_433d599dce005.tif
PAX_Noc30_433d599dce006.tif
PAX_Noc30_433d599dce007.tif
PAX_Noc30_433d599dce008.tif
PAX_Noc30_433d599dce009.tif
PAX_Noc30_433d599dce010.tif
PAX_Noc30_433d599dce011.tif
PAX_Noc30_433d599dce012.tif
PAX_Noc30_433d599dce013.tif

Nom du fichier :

Fichiers de type : Tous les fichiers (*.*)

Ouvrir

Annuler



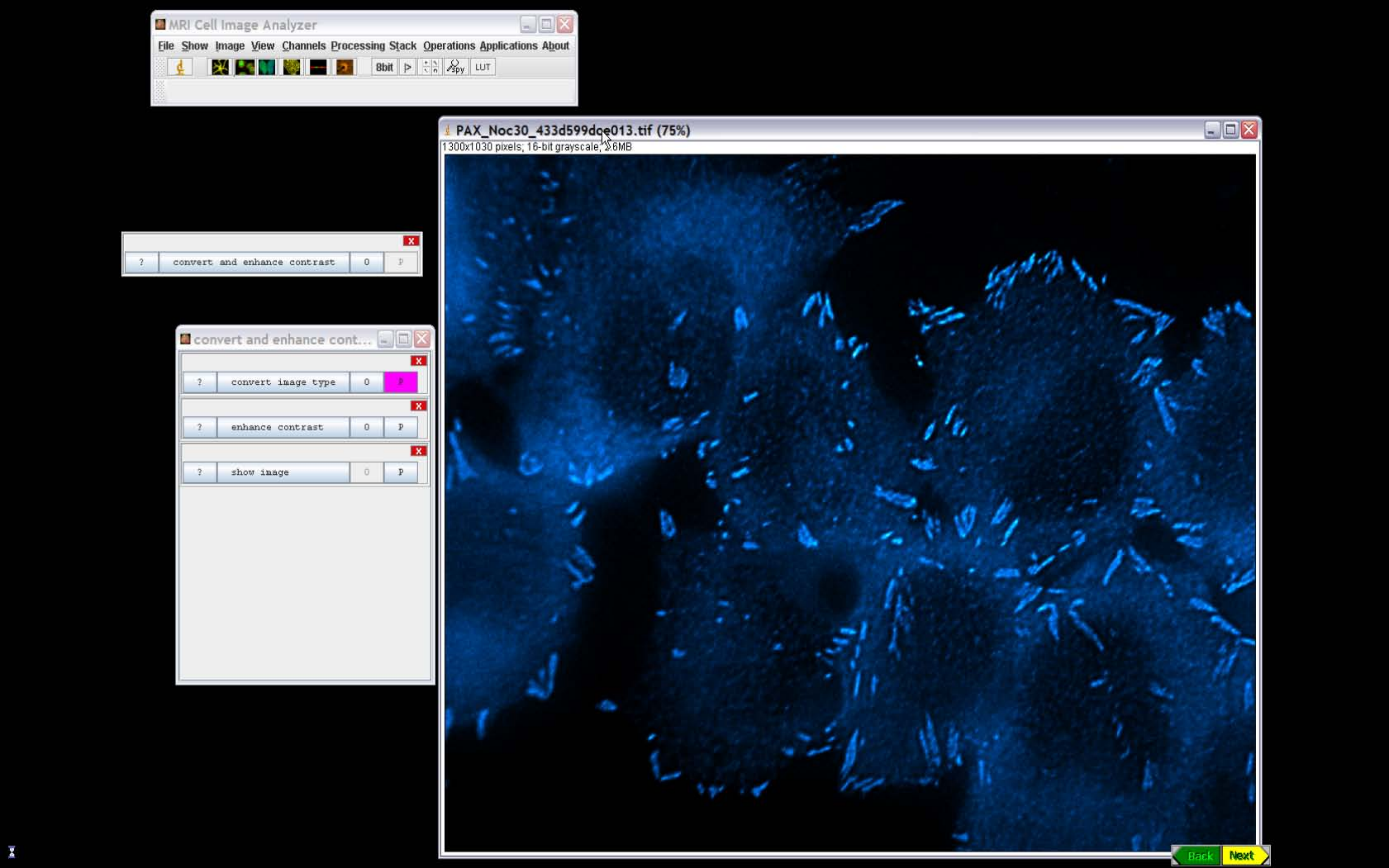
x=1224, y=468, value=21

Regarder dans : Pax-Nocodazole

PAX_Noc30_433d599dce000.tif
PAX_Noc30_433d599dce001.tif
PAX_Noc30_433d599dce002.tif
PAX_Noc30_433d599dce003.tif
PAX_Noc30_433d599dce004.tif
PAX_Noc30_433d599dce005.tif
PAX_Noc30_433d599dce006.tif
PAX_Noc30_433d599dce007.tif
PAX_Noc30_433d599dce008.tif
PAX_Noc30_433d599dce009.tif
PAX_Noc30_433d599dce010.tif
PAX_Noc30_433d599dce011.tif
PAX_Noc30_433d599dce012.tif
PAX_Noc30_433d599dce013.tif

Nom du fichier : PAX_Noc30_433d599dce013.tif

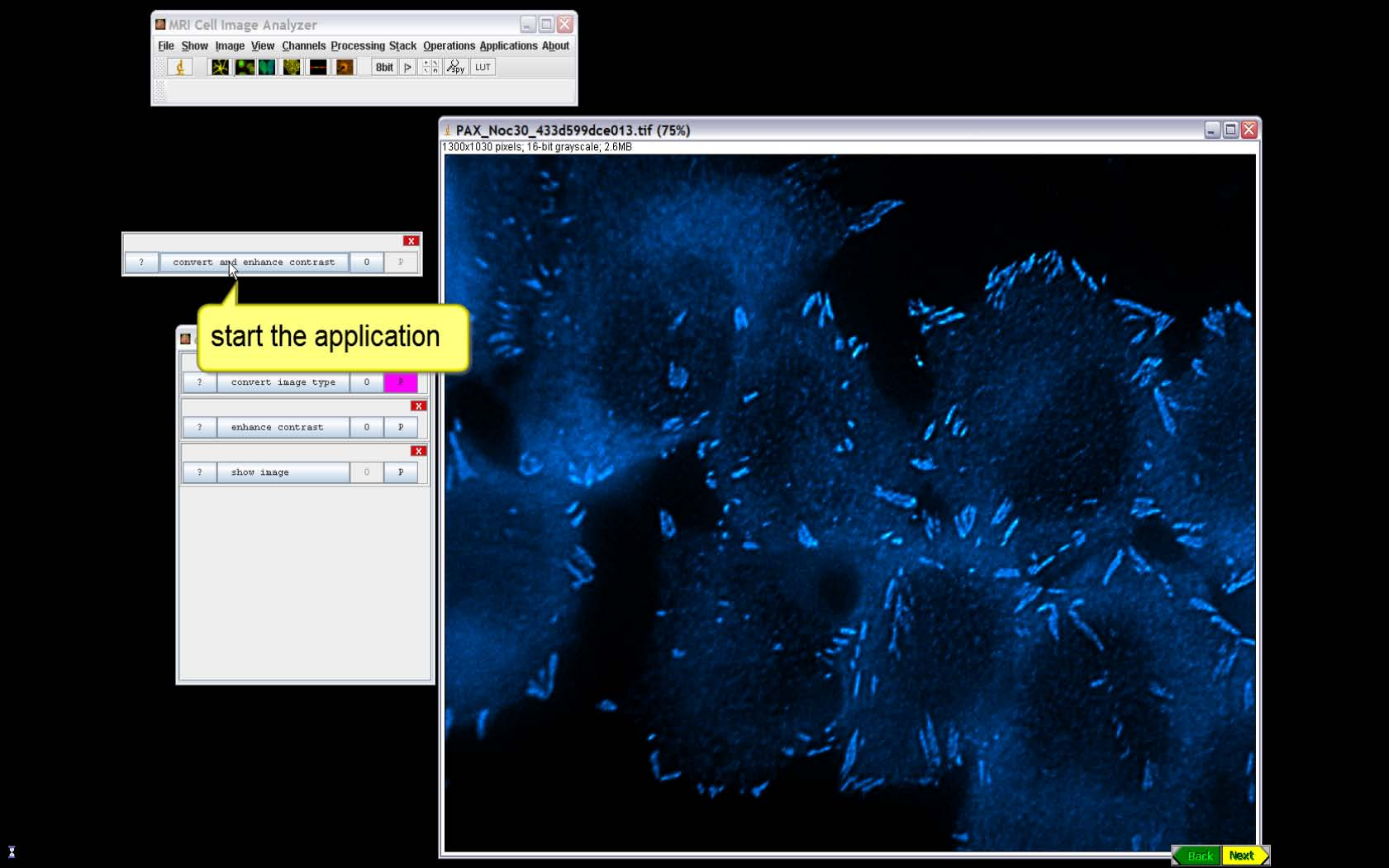
Fichiers de type : Tous les fichiers (*.*)



MRI Cell Image Analyzer
File Show Image View Channels Processing Stack Operations Applications About
8bit
spy
LUT
?
convert and enhance contrast
0
P
convert and enhance cont...
?
convert image type
0
P
?
enhance contrast
0
P
?
show image
0
P
Back
Next

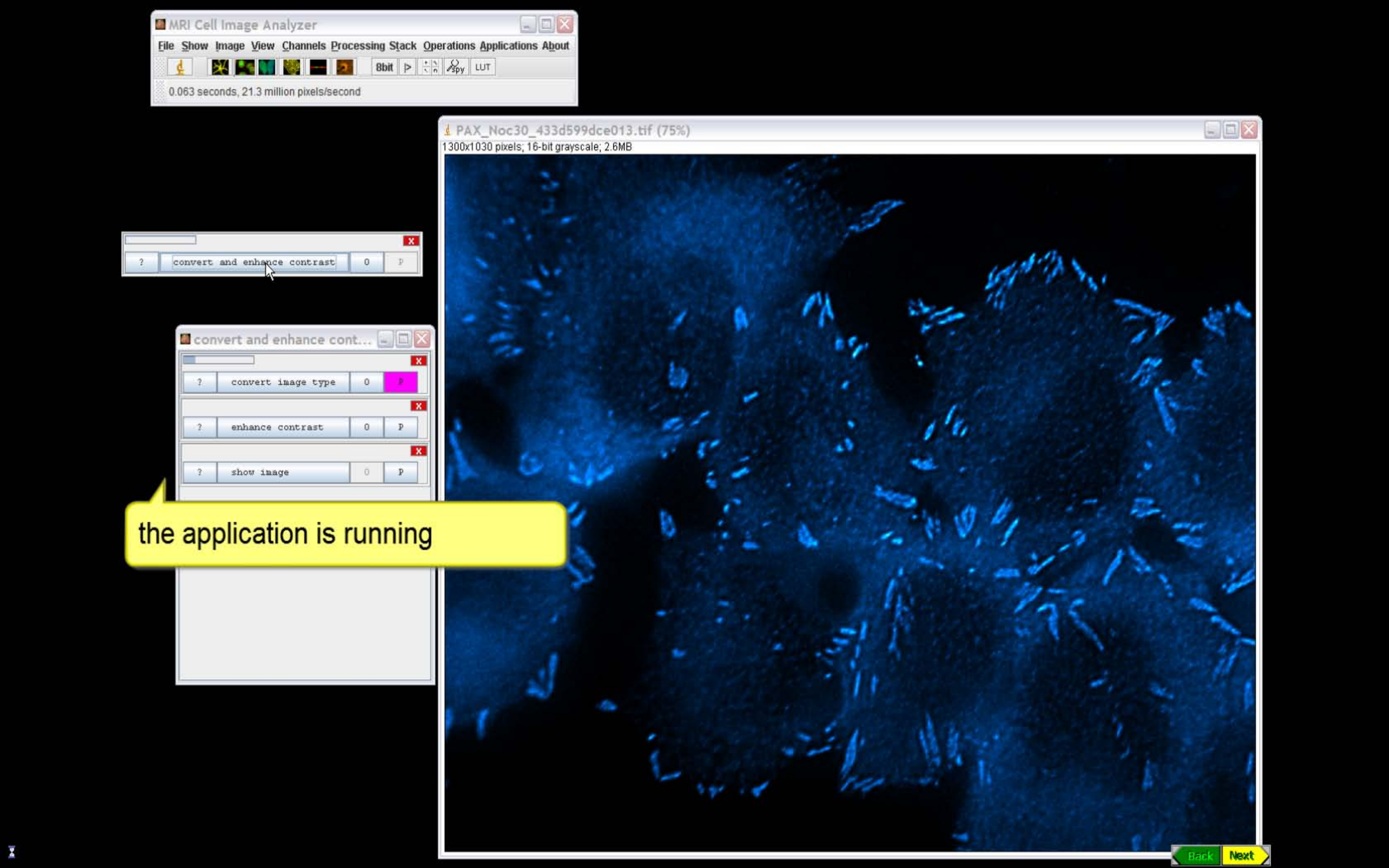
PAX_Noc30_433d599dqe013.tif (75%)

1300x1030 pixels; 16-bit grayscale; 2.6MB


MRI Cell Image Analyzer
File Show Image View Channels Processing Stack Operations Applications About
8bit
LUT
? convert and enhance contrast 0 P
? convert image type 0 P
? enhance contrast 0 P
? show image 0 P
PAX_Noc30_433d599dce013.tif (75%)
Back
Next

1300x1030 pixels; 16-bit grayscale; 2.6MB

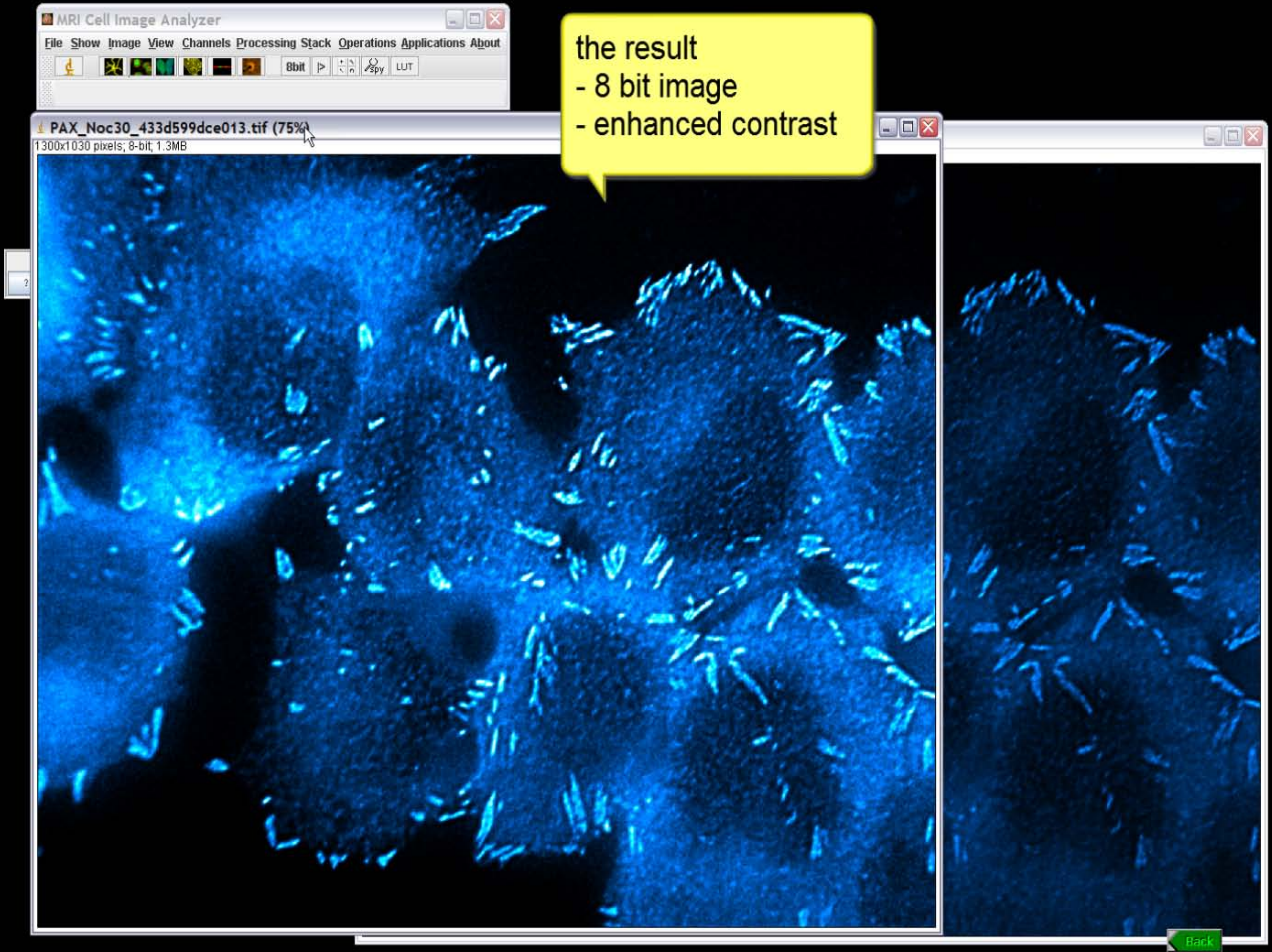
start the application


MRI Cell Image Analyzer
File Show Image View Channels Processing Stack Operations Applications About
8bit
LUT
PAX_Noc30_433d599dce013.tif (75%)
?
convert and enhance contrast
0
P
convert and enhance cont...
convert image type
enhance contrast
show image
Back
Next

0.063 seconds, 21.3 million pixels/second

1300x1030 pixels; 16-bit grayscale; 2.6MB

the application is running

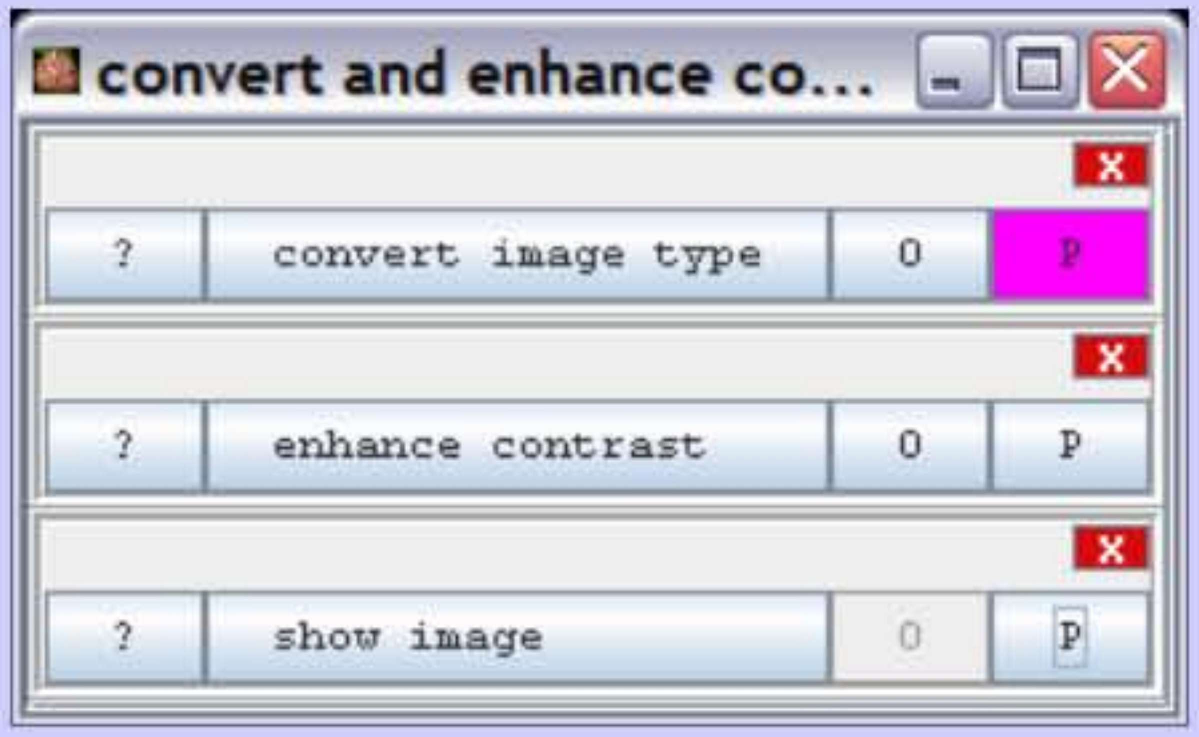

MRI Cell Image Analyzer
File Show Image View Channels Processing Stack Operations Applications About
8bit
LUT
PAX_Noc30_433d599dce013.tif (75%)
Back

1300x1030 pixels; 8-bit; 1.3MB

the result
- 8 bit image
- enhanced contrast

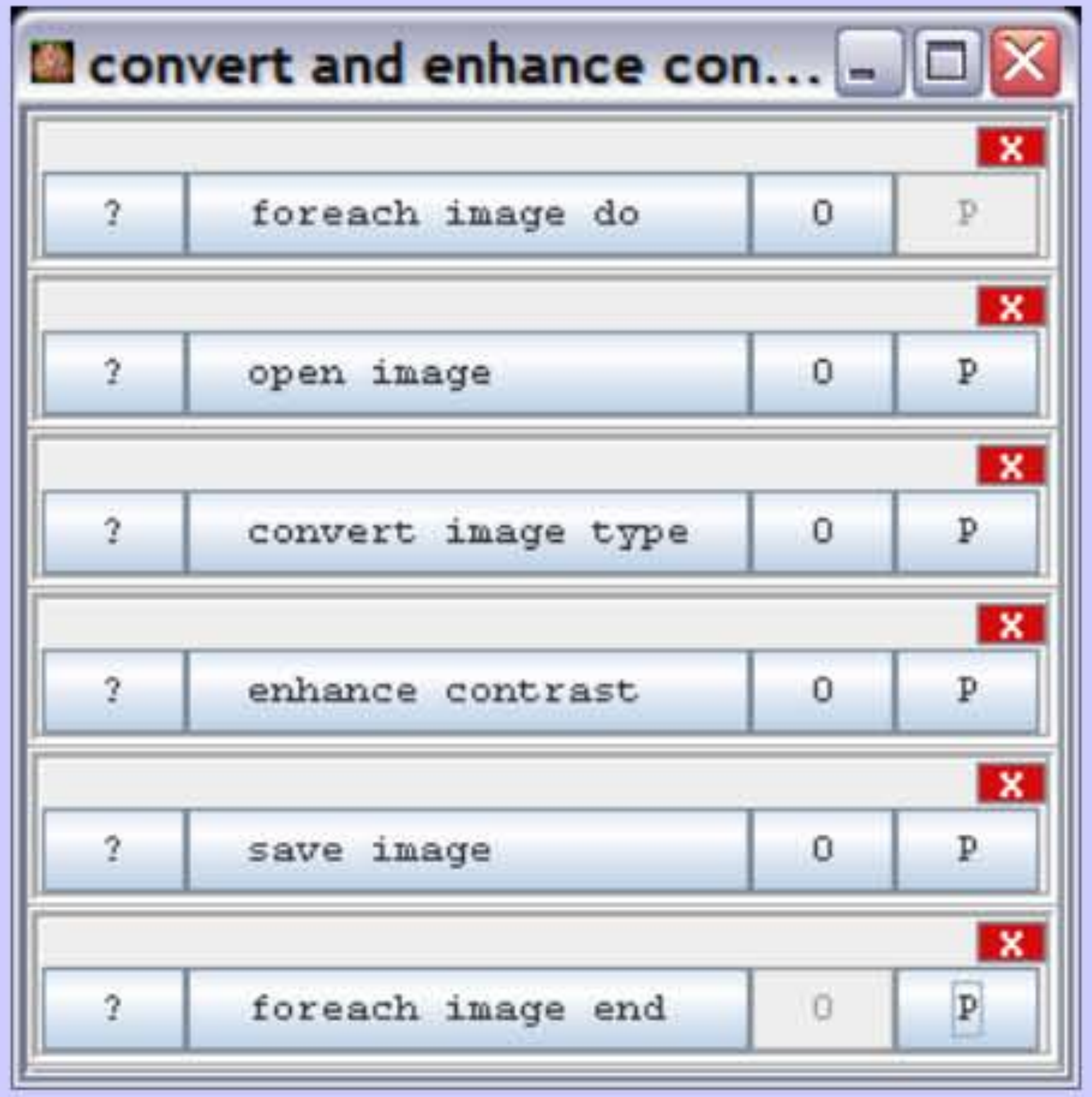
Using visual scripting

- create a batch application



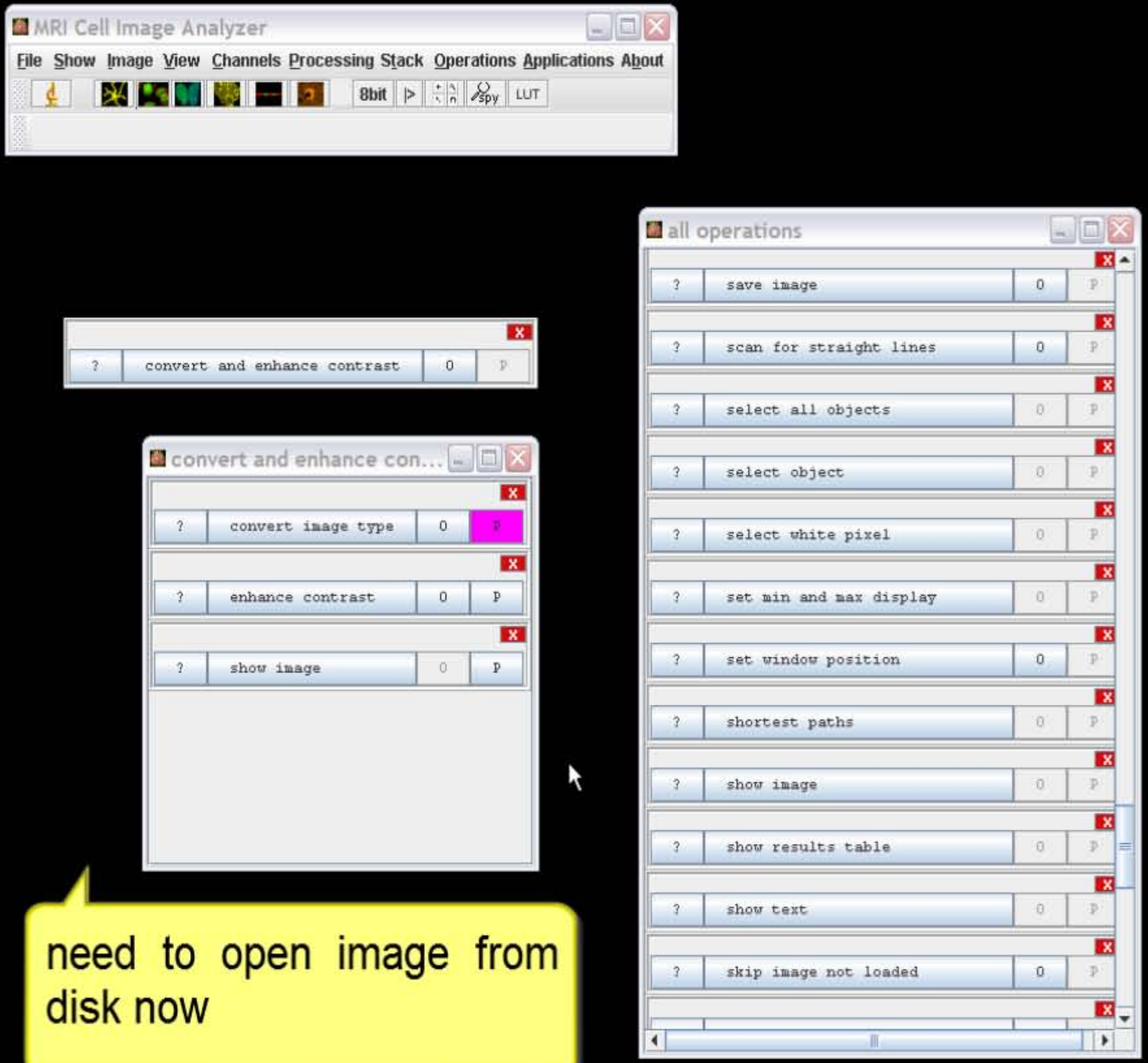
convert and enhance co...

?	convert image type	0	P
?	enhance contrast	0	P
?	show image	0	P



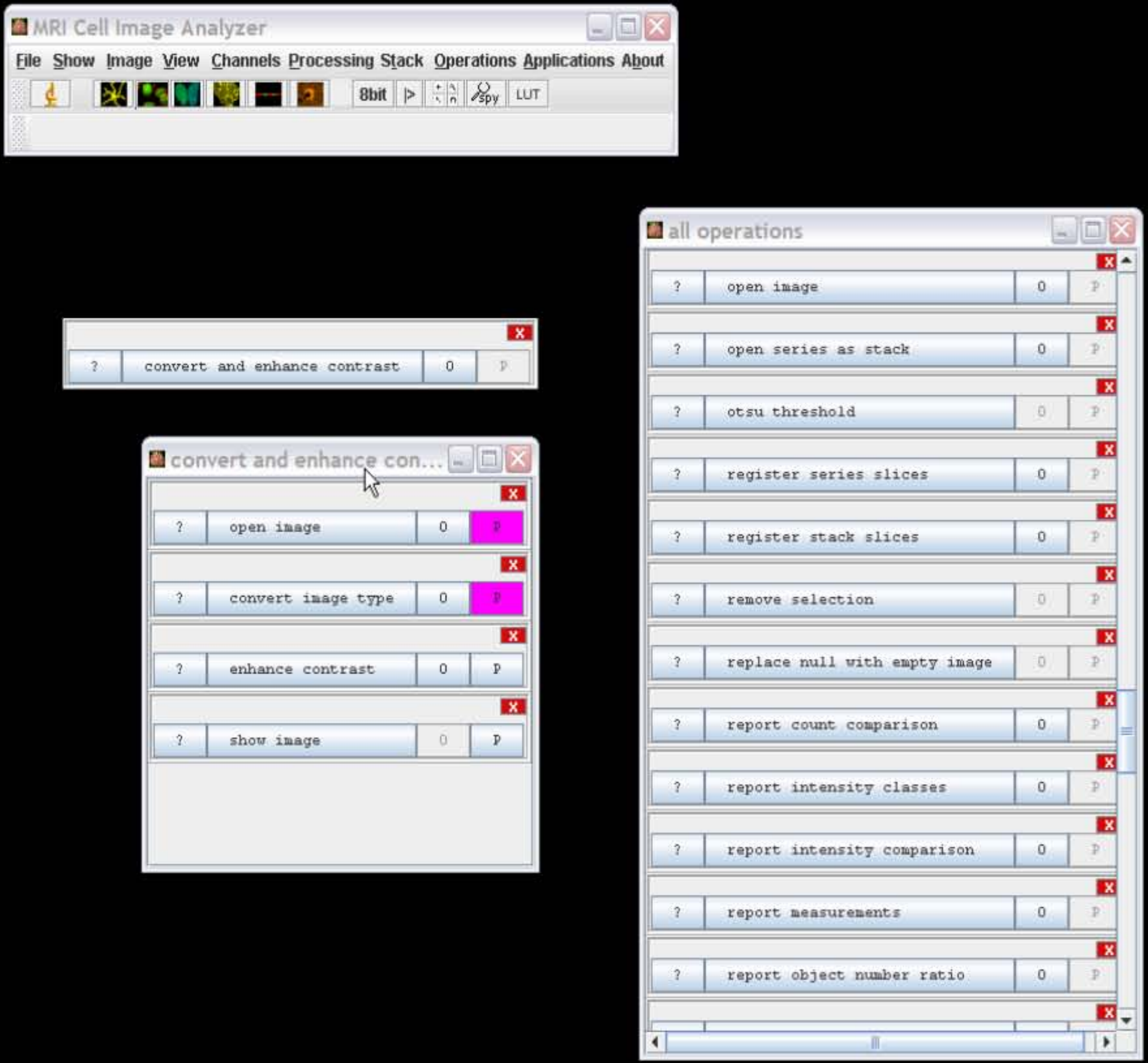
convert and enhance con...

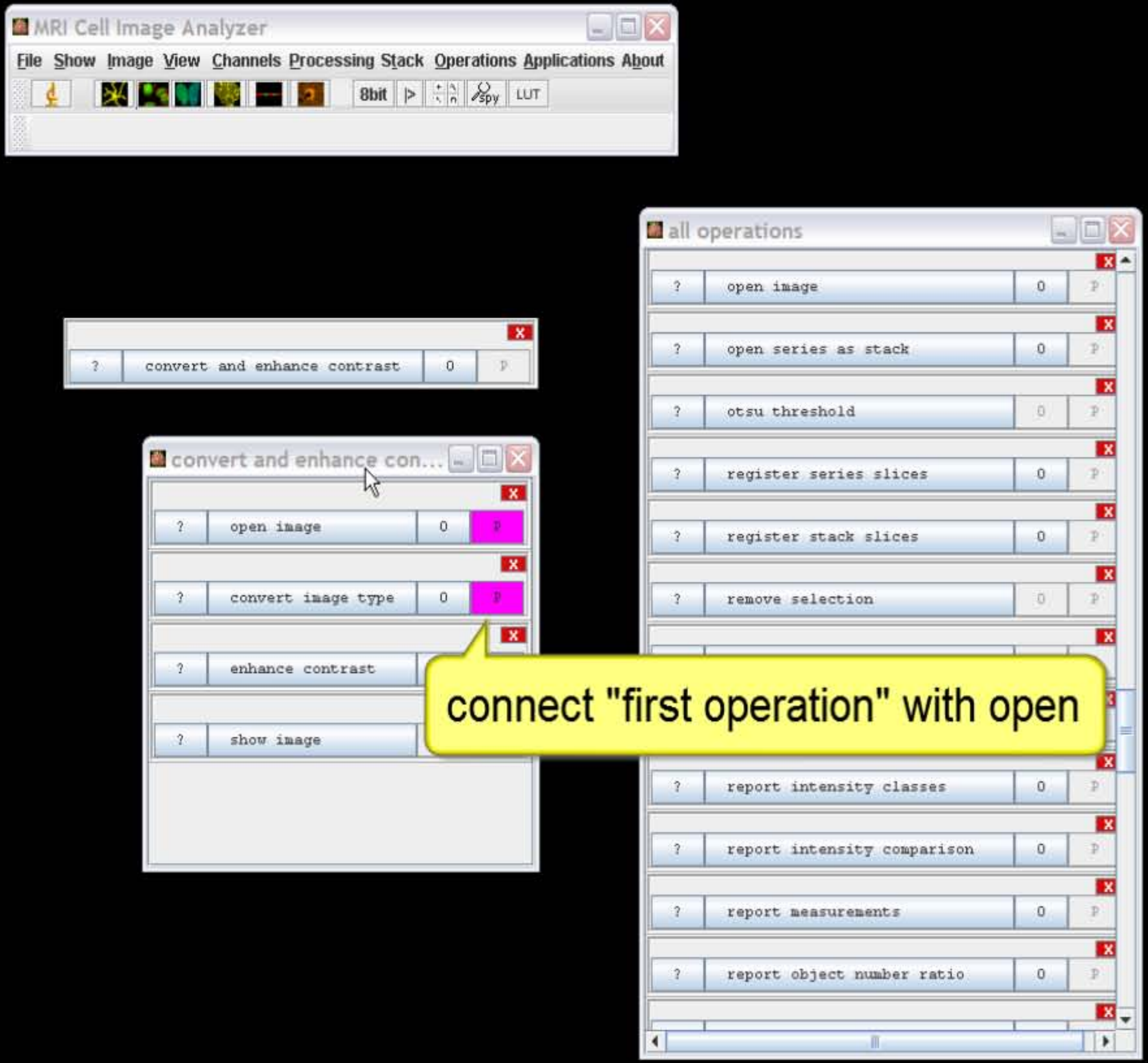
?	foreach image do	0	P
?	open image	0	P
?	convert image type	0	P
?	enhance contrast	0	P
?	save image	0	P
?	foreach image end	0	P



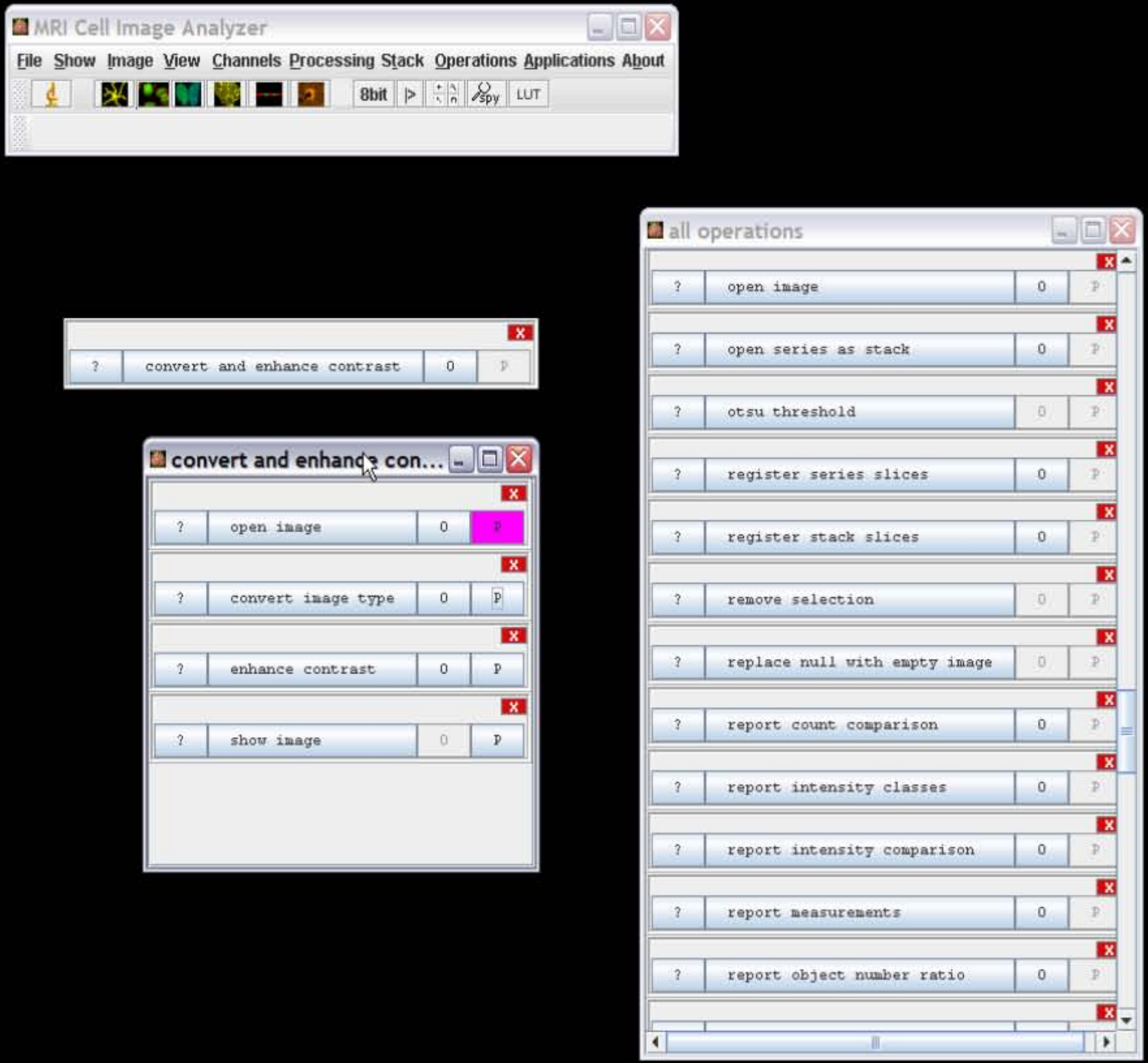
need to open image from disk now

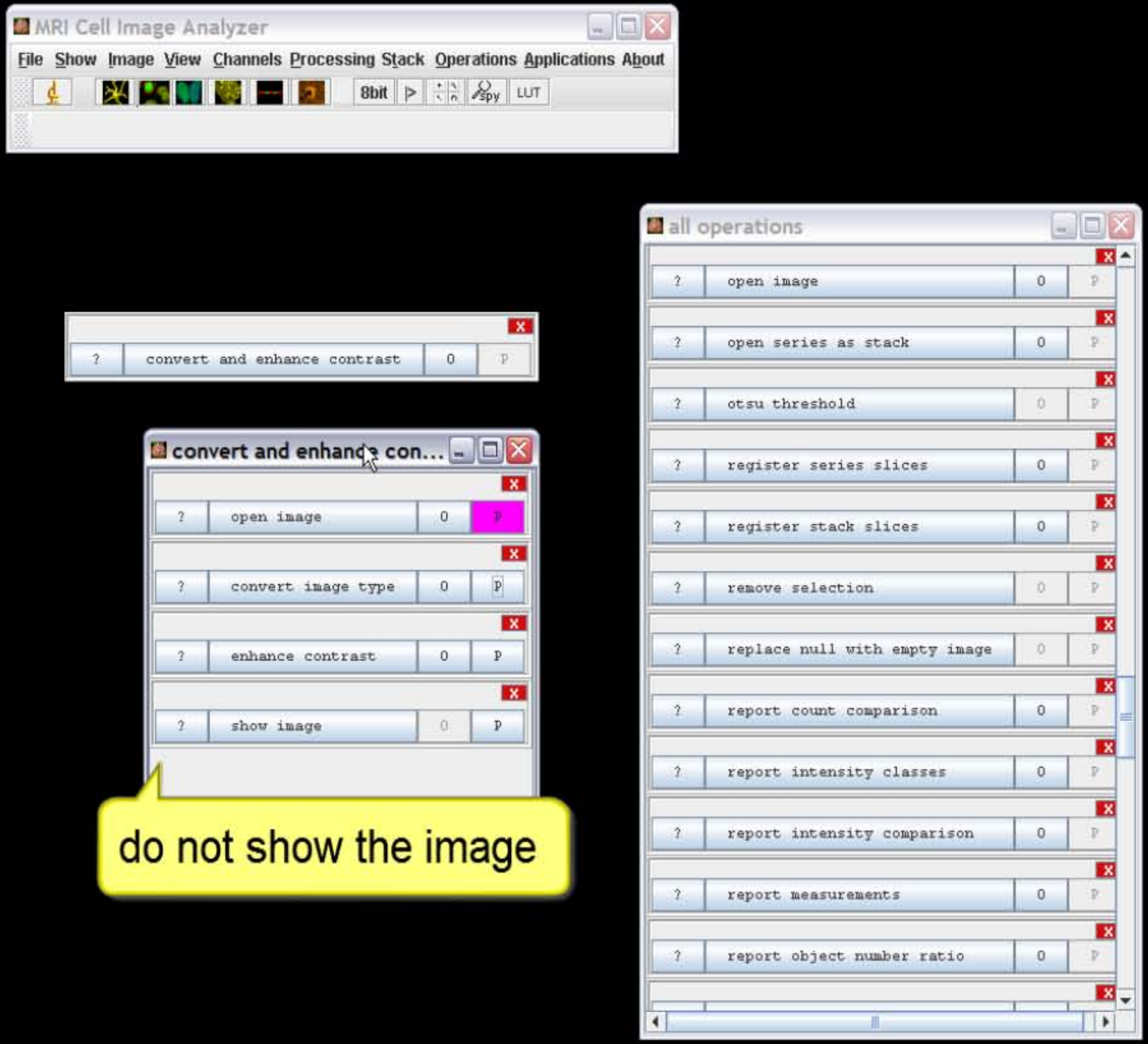
Next


MRI Cell Image Analyzer
File Show Image View Channels Processing Stack Operations Applications About
8bit
LUT
? convert and enhance contrast 0 P
convert and enhance con...
? open image 0 P
? convert image type 0 P
? enhance contrast 0 P
? show image 0 P
all operations
? open image 0 P
? open series as stack 0 P
? otsu threshold 0 P
? register series slices 0 P
? register stack slices 0 P
? remove selection 0 P
? replace null with empty image 0 P
? report count comparison 0 P
? report intensity classes 0 P
? report intensity comparison 0 P
? report measurements 0 P
? report object number ratio 0 P


MRI Cell Image Analyzer
File Show Image View Channels Processing Stack Operations Applications About
8bit
LUT
? convert and enhance contrast 0 P
convert and enhance con...
? open image 0 P
? convert image type 0 P
? enhance contrast
? show image
all operations
? open image 0 P
? open series as stack 0 P
? otsu threshold 0 P
? register series slices 0 P
? register stack slices 0 P
? remove selection 0 P
? report intensity classes 0 P
? report intensity comparison 0 P
? report measurements 0 P
? report object number ratio 0 P

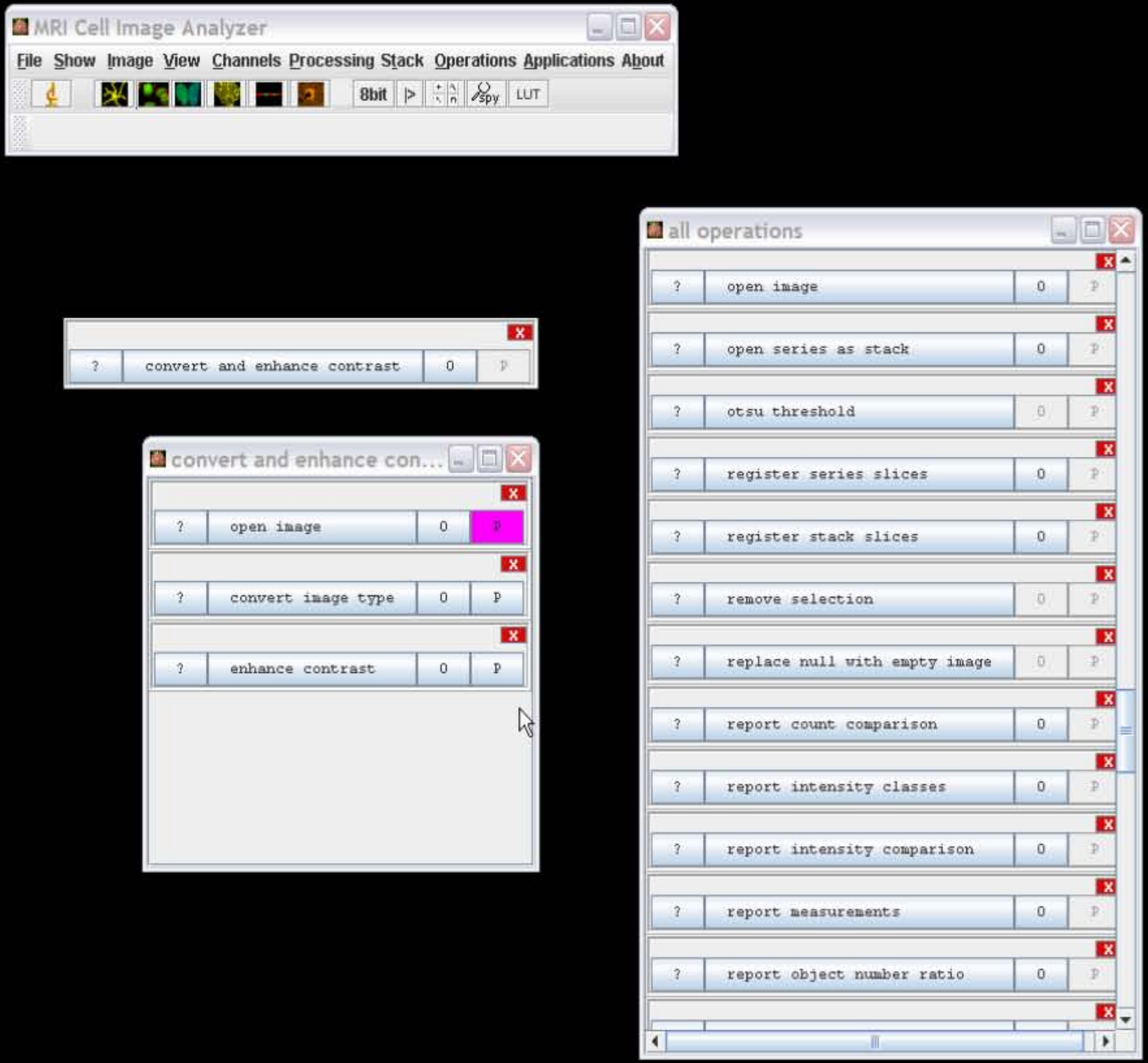
connect "first operation" with open

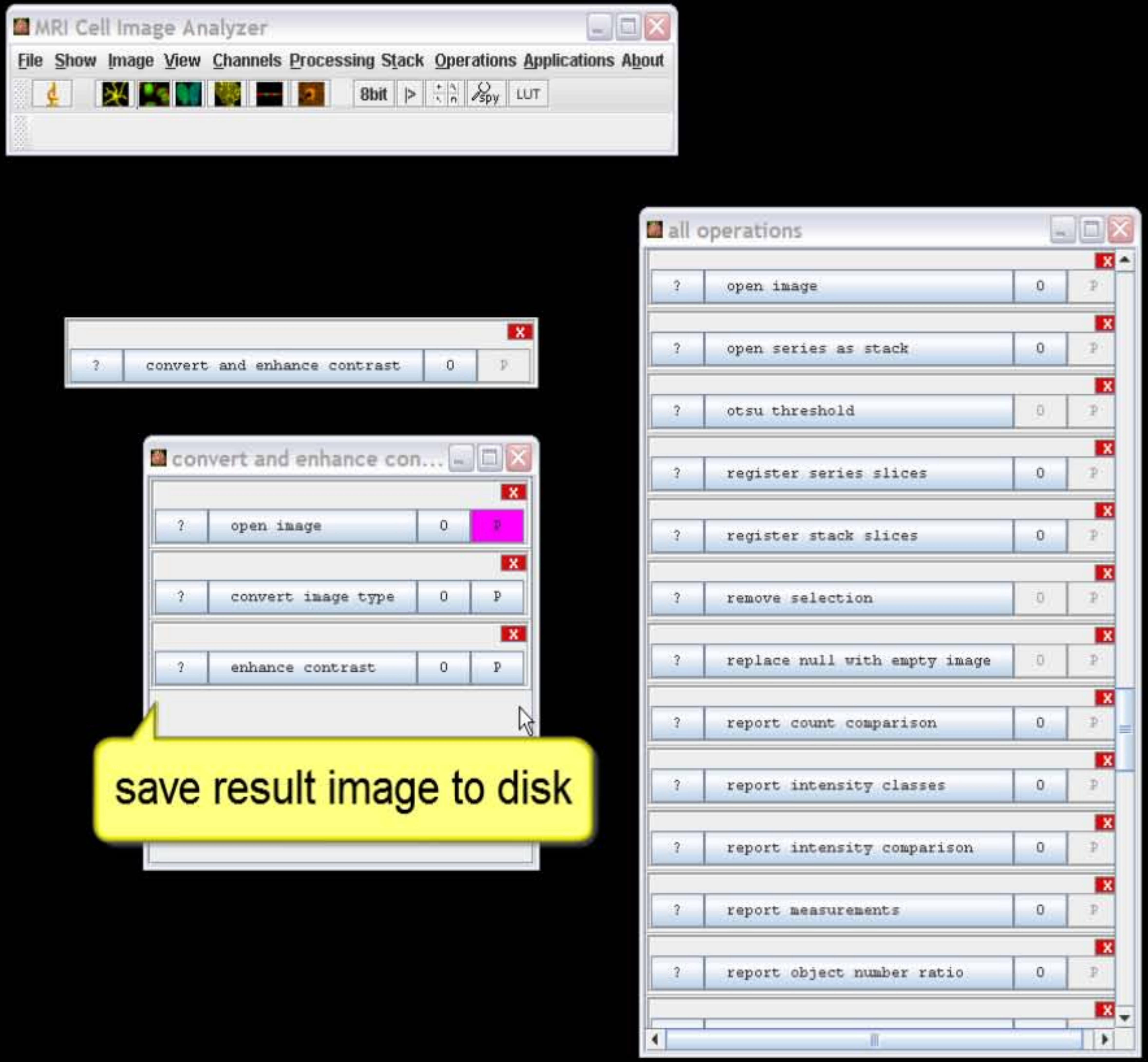

MRI Cell Image Analyzer
File Show Image View Channels Processing Stack Operations Applications About
8bit
spy
LUT
? convert and enhance contrast 0 P
convert and enhance con...
? open image 0 P
? convert image type 0 P
? enhance contrast 0 P
? show image 0 P
all operations
? open image 0 P
? open series as stack 0 P
? otsu threshold 0 P
? register series slices 0 P
? register stack slices 0 P
? remove selection 0 P
? replace null with empty image 0 P
? report count comparison 0 P
? report intensity classes 0 P
? report intensity comparison 0 P
? report measurements 0 P
? report object number ratio 0 P


MRI Cell Image Analyzer
File Show Image View Channels Processing Stack Operations Applications About
8bit
LUT
? convert and enhance contrast 0 P
convert and enhance con...
? open image 0 P
? convert image type 0 P
? enhance contrast 0 P
? show image 0 P
all operations
? open image 0 P
? open series as stack 0 P
? otsu threshold 0 P
? register series slices 0 P
? register stack slices 0 P
? remove selection 0 P
? replace null with empty image 0 P
? report count comparison 0 P
? report intensity classes 0 P
? report intensity comparison 0 P
? report measurements 0 P
? report object number ratio 0 P

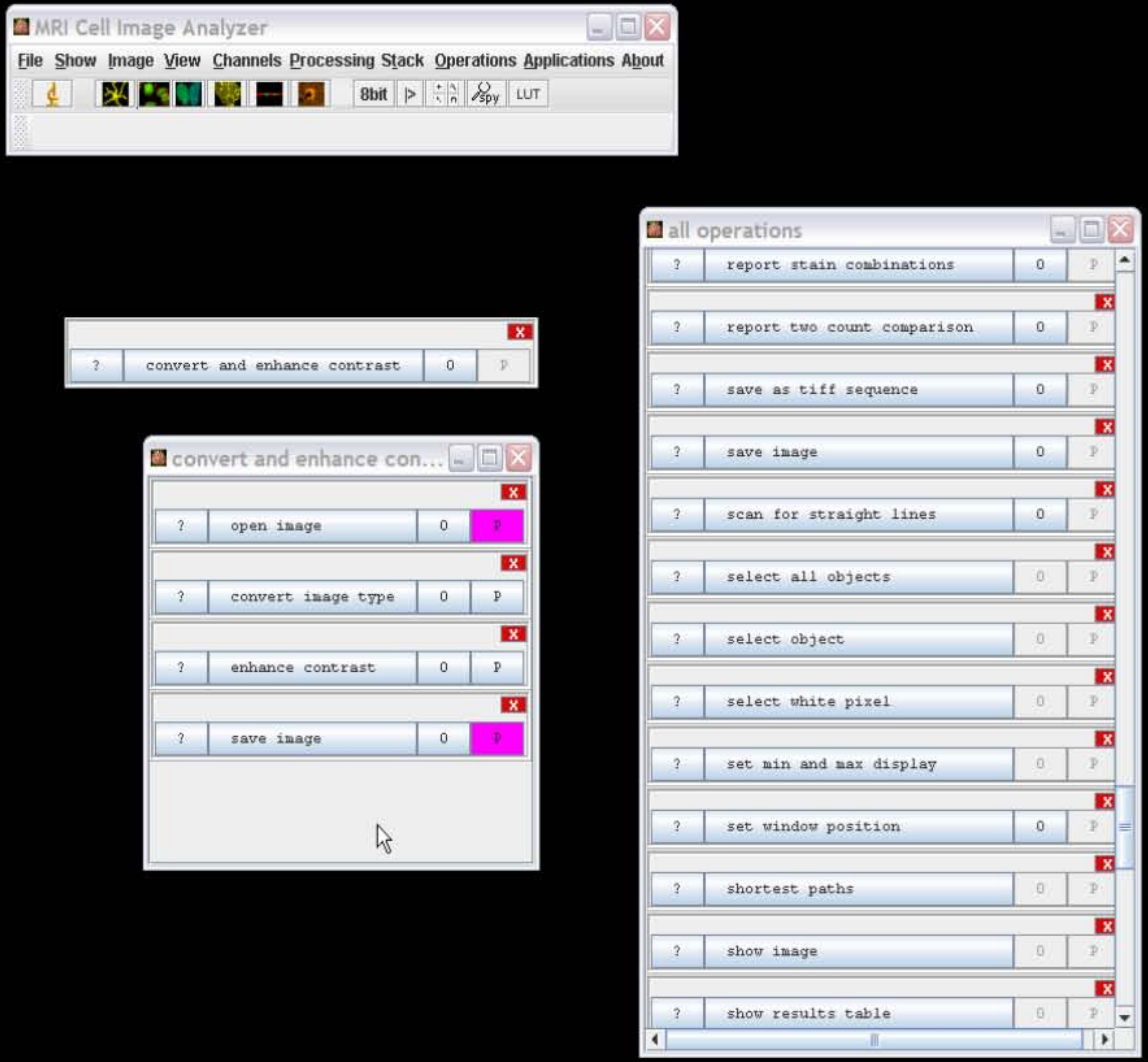
do not show the image

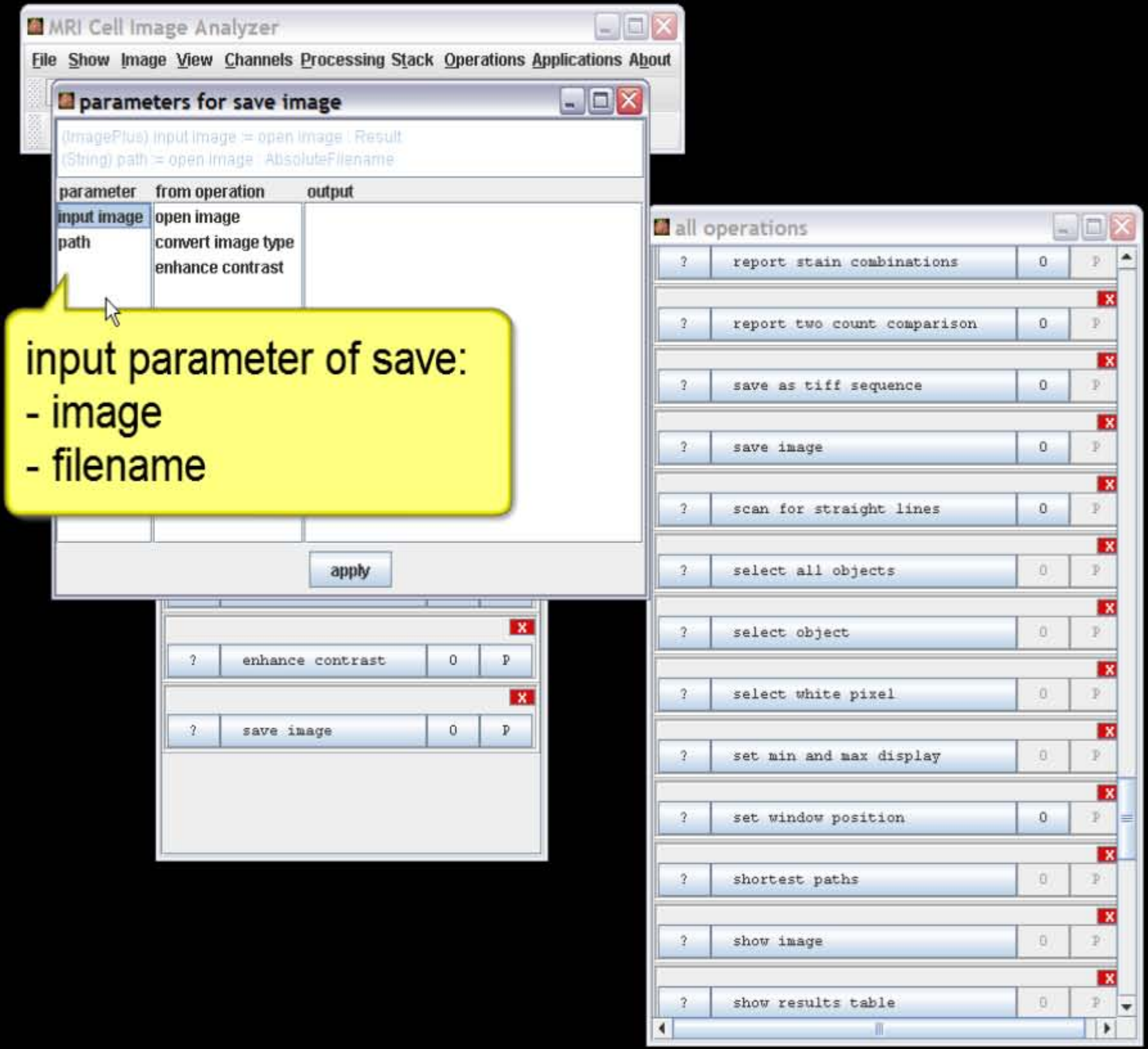
Back Next


MRI Cell Image Analyzer
File Show Image View Channels Processing Stack Operations Applications About
8bit
spy
LUT
? convert and enhance contrast 0 P
convert and enhance con...
? open image 0 P
? convert image type 0 P
? enhance contrast 0 P
all operations
? open image 0 P
? open series as stack 0 P
? otsu threshold 0 P
? register series slices 0 P
? register stack slices 0 P
? remove selection 0 P
? replace null with empty image 0 P
? report count comparison 0 P
? report intensity classes 0 P
? report intensity comparison 0 P
? report measurements 0 P
? report object number ratio 0 P


MRI Cell Image Analyzer
File Show Image View Channels Processing Stack Operations Applications About
8bit LUT
? convert and enhance contrast 0 P
convert and enhance con...
? open image 0 P
? convert image type 0 P
? enhance contrast 0 P
all operations
? open image 0 P
? open series as stack 0 P
? otsu threshold 0 P
? register series slices 0 P
? register stack slices 0 P
? remove selection 0 P
? replace null with empty image 0 P
? report count comparison 0 P
? report intensity classes 0 P
? report intensity comparison 0 P
? report measurements 0 P
? report object number ratio 0 P

save result image to disk


MRI Cell Image Analyzer
File Show Image View Channels Processing Stack Operations Applications About
8bit
LUT
? convert and enhance contrast 0 P
convert and enhance con...
? open image 0 P
? convert image type 0 P
? enhance contrast 0 P
? save image 0 P
all operations
? report stain combinations 0 P
? report two count comparison 0 P
? save as tiff sequence 0 P
? save image 0 P
? scan for straight lines 0 P
? select all objects 0 P
? select object 0 P
? select white pixel 0 P
? set min and max display 0 P
? set window position 0 P
? shortest paths 0 P
? show image 0 P
? show results table 0 P


MRI Cell Image Analyzer
File Show Image View Channels Processing Stack Operations Applications About
parameters for save image
apply
all operations

(ImagePlus) input image := open image : Result
(String) path := open image : AbsoluteFilename

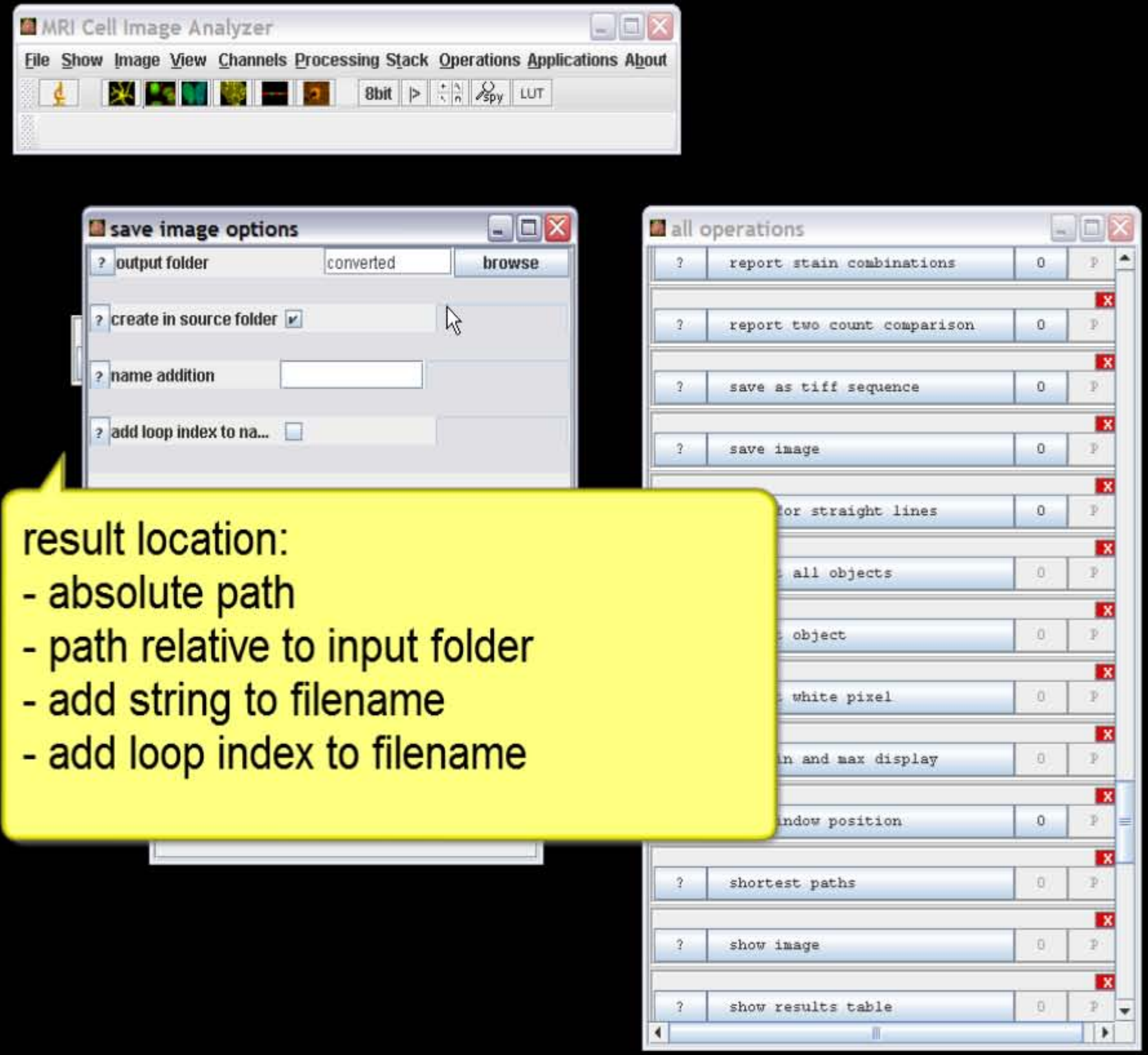
parameter	from operation	output
input image	open image	
path	convert image type	
	enhance contrast	

```
?  enhance contrast   0  P
?  save image         0  P
```

```
?  report stain combinations       0  P
?  report two count comparison     0  P
?  save as tiff sequence           0  P
?  save image                      0  P
?  scan for straight lines         0  P
?  select all objects              0  P
?  select object                   0  P
?  select white pixel              0  P
?  set min and max display         0  P
?  set window position             0  P
?  shortest paths                  0  P
?  show image                      0  P
?  show results table              0  P
```

input parameter of save:
- image
- filename

Back Next



save image options

output folder: converted — browse

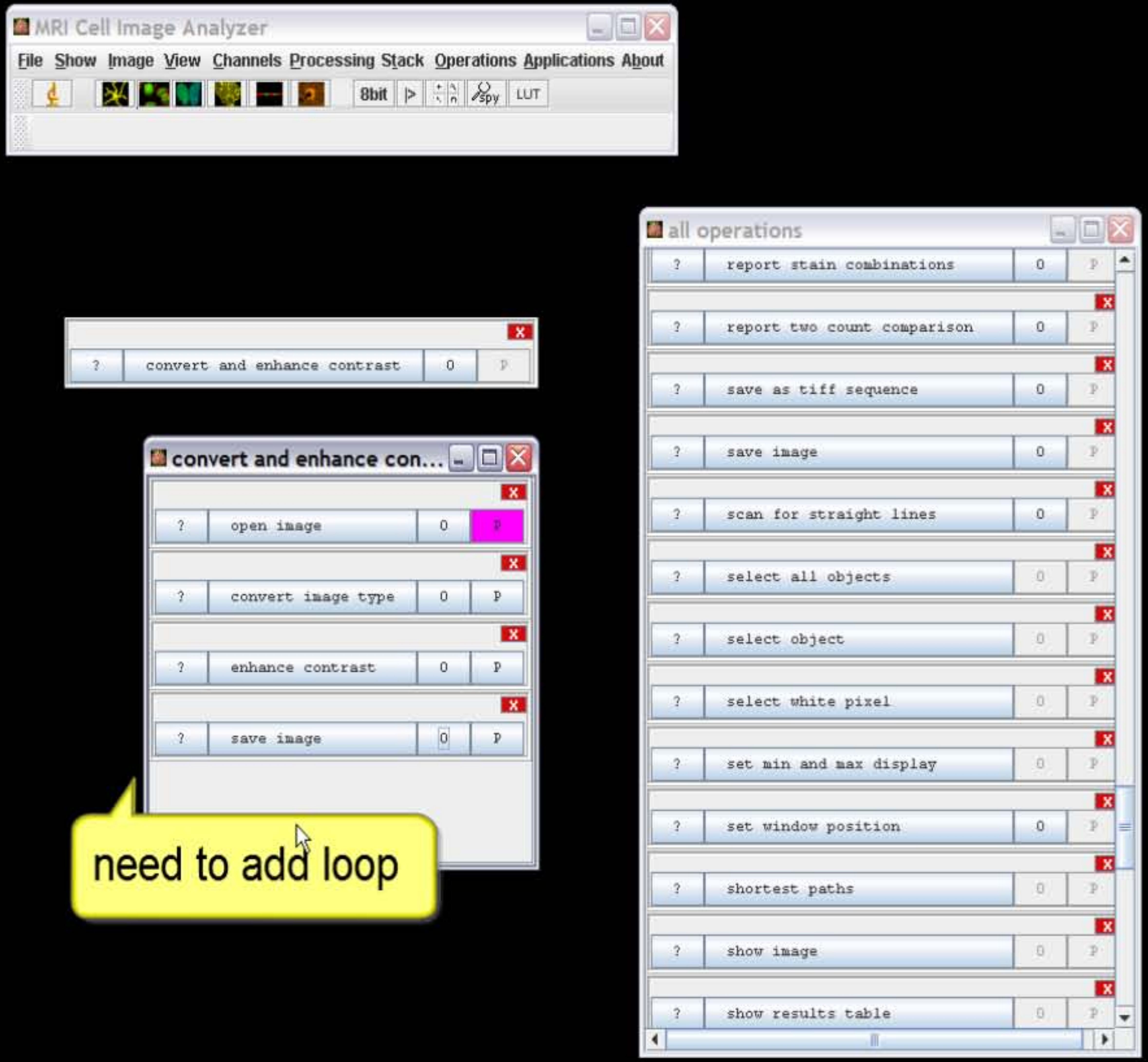
create in source folder ☑

name addition:

add loop index to na... ☐

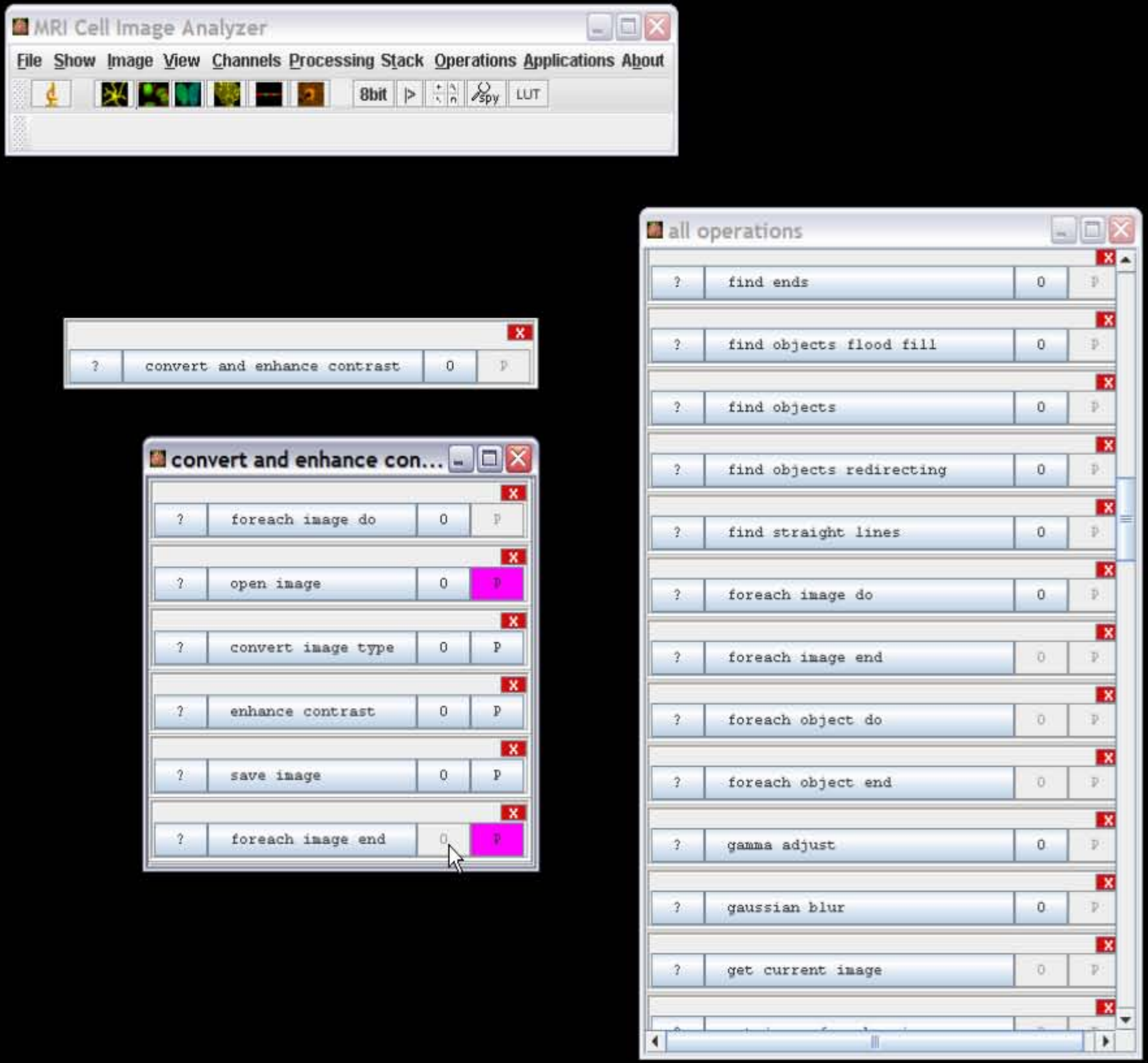
result location:
- absolute path
- path relative to input folder
- add string to filename
- add loop index to filename

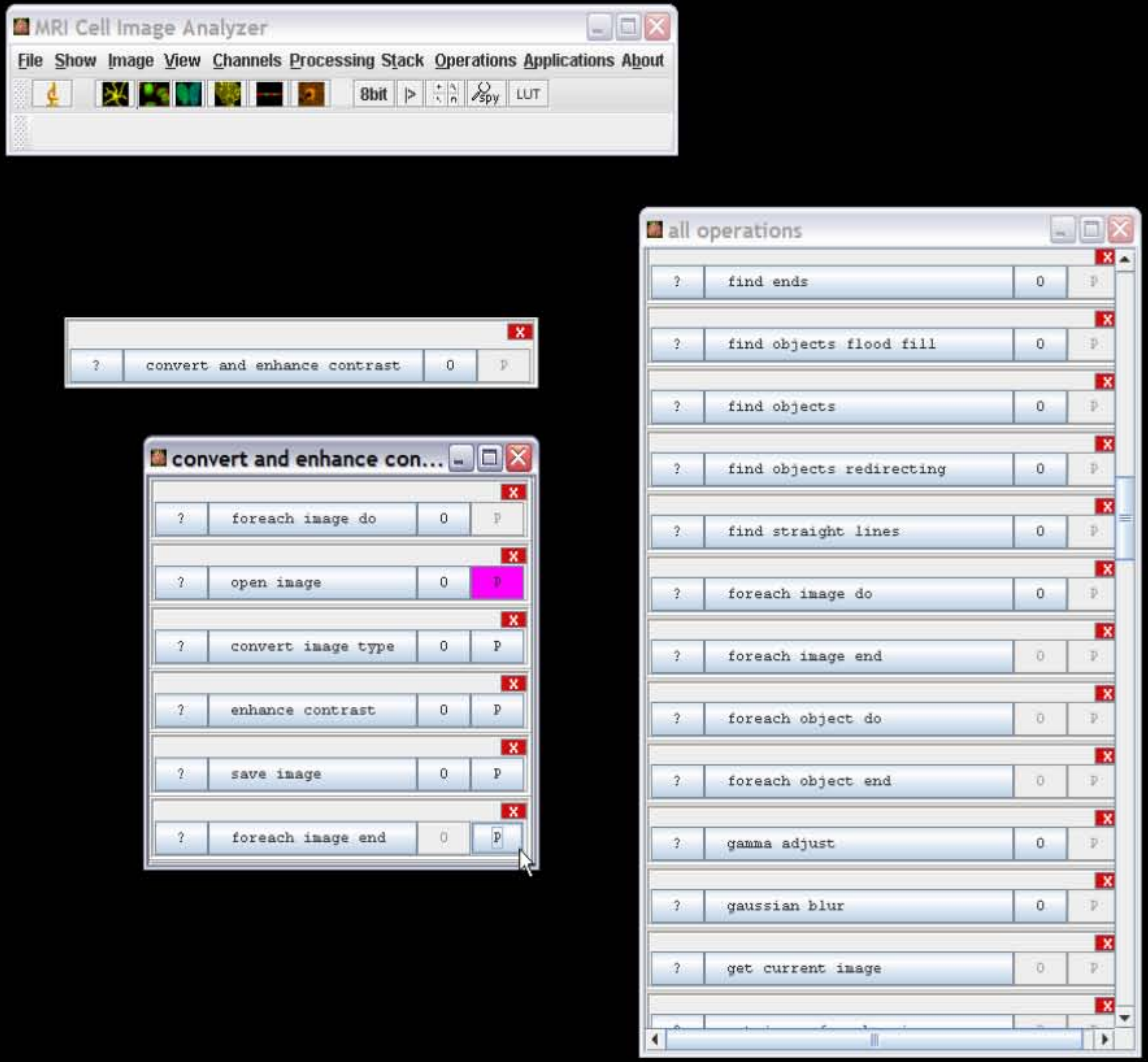
Back Next

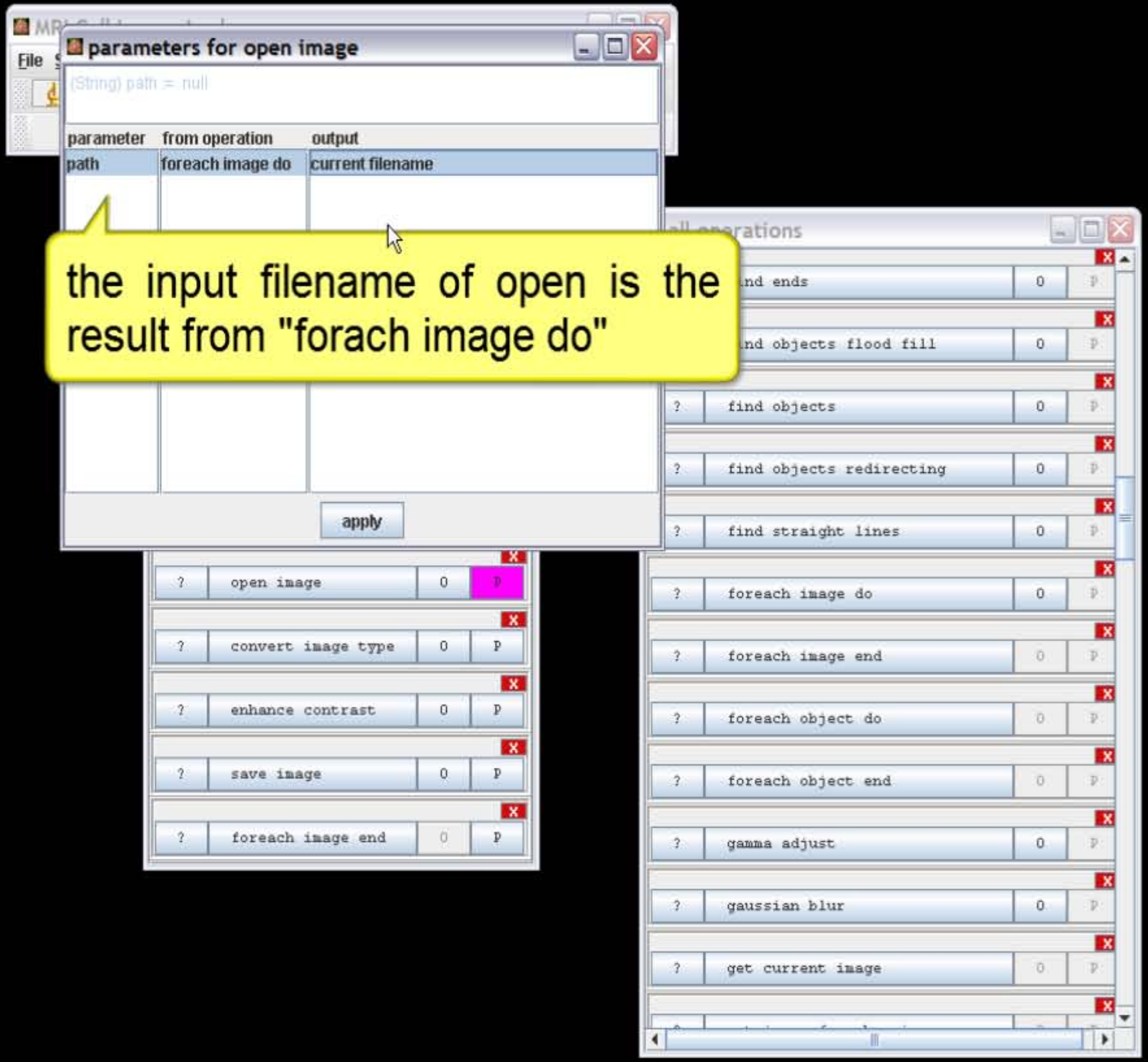

MRI Cell Image Analyzer
File Show Image View Channels Processing Stack Operations Applications About
8bit
spy
LUT
? convert and enhance contrast 0 P
convert and enhance con...
? open image 0 P
? convert image type 0 P
? enhance contrast 0 P
? save image 0 P
all operations
? report stain combinations 0 P
? report two count comparison 0 P
? save as tiff sequence 0 P
? save image 0 P
? scan for straight lines 0 P
? select all objects 0 P
? select object 0 P
? select white pixel 0 P
? set min and max display 0 P
? set window position 0 P
? shortest paths 0 P
? show image 0 P
? show results table 0 P

need to add loop

Back Next


MRI Cell Image Analyzer
File Show Image View Channels Processing Stack Operations Applications About
8bit
LUT
? convert and enhance contrast 0 P
convert and enhance con...
? foreach image do 0 P
? open image 0 P
? convert image type 0 P
? enhance contrast 0 P
? save image 0 P
? foreach image end 0 P
all operations
? find ends 0 P
? find objects flood fill 0 P
? find objects 0 P
? find objects redirecting 0 P
? find straight lines 0 P
? foreach image do 0 P
? foreach image end 0 P
? foreach object do 0 P
? foreach object end 0 P
? gamma adjust 0 P
? gaussian blur 0 P
? get current image 0 P


MRI Cell Image Analyzer
File Show Image View Channels Processing Stack Operations Applications About
8bit
LUT
? convert and enhance contrast 0 P
convert and enhance con...
? foreach image do 0 P
? open image 0 P
? convert image type 0 P
? enhance contrast 0 P
? save image 0 P
? foreach image end 0 P
all operations
? find ends 0 P
? find objects flood fill 0 P
? find objects 0 P
? find objects redirecting 0 P
? find straight lines 0 P
? foreach image do 0 P
? foreach image end 0 P
? foreach object do 0 P
? foreach object end 0 P
? gamma adjust 0 P
? gaussian blur 0 P
? get current image 0 P



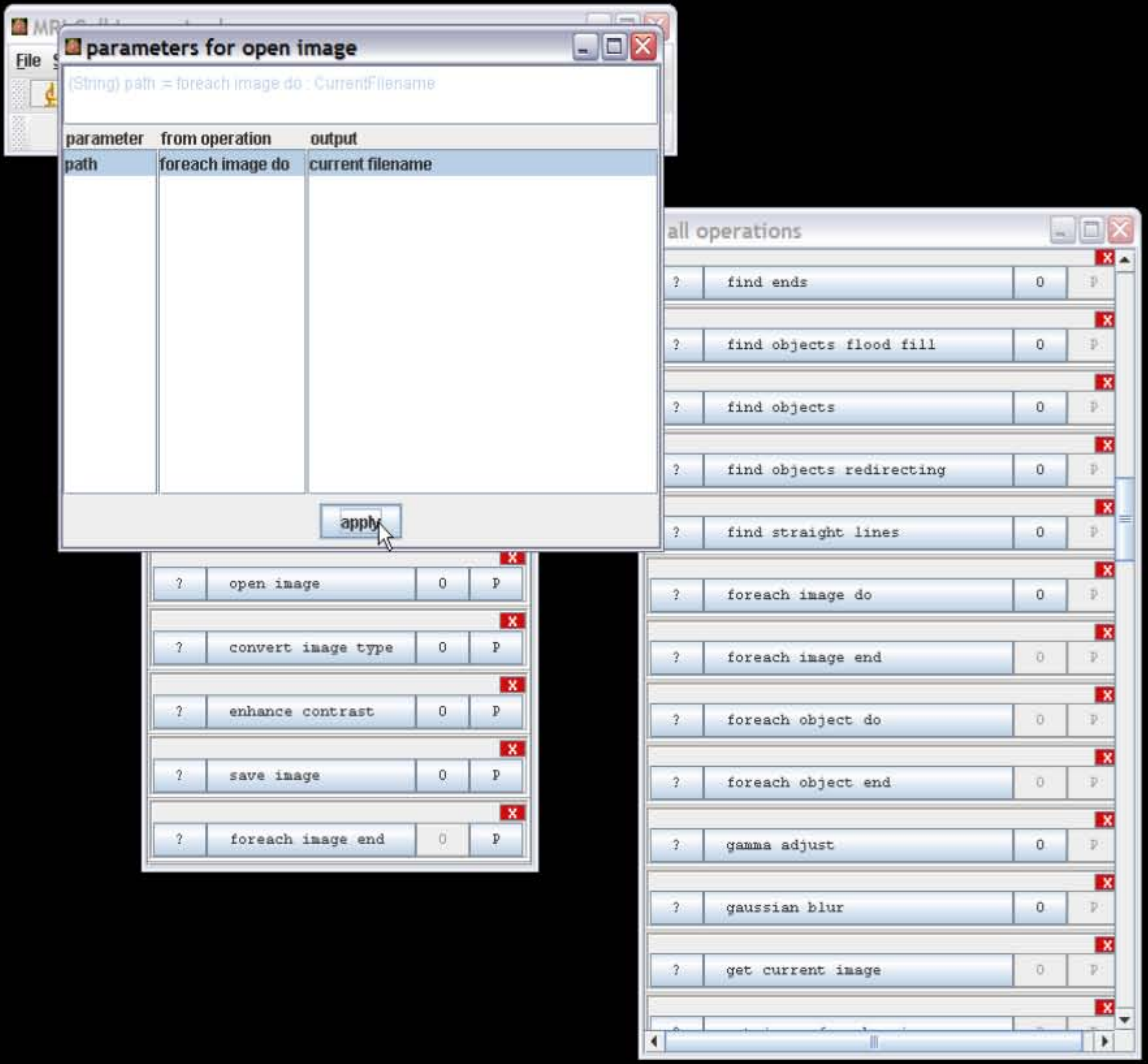
parameters for open image

(String) path = null

parameter	from operation	output
path	foreach image do	current filename

the input filename of open is the result from "forach image do"

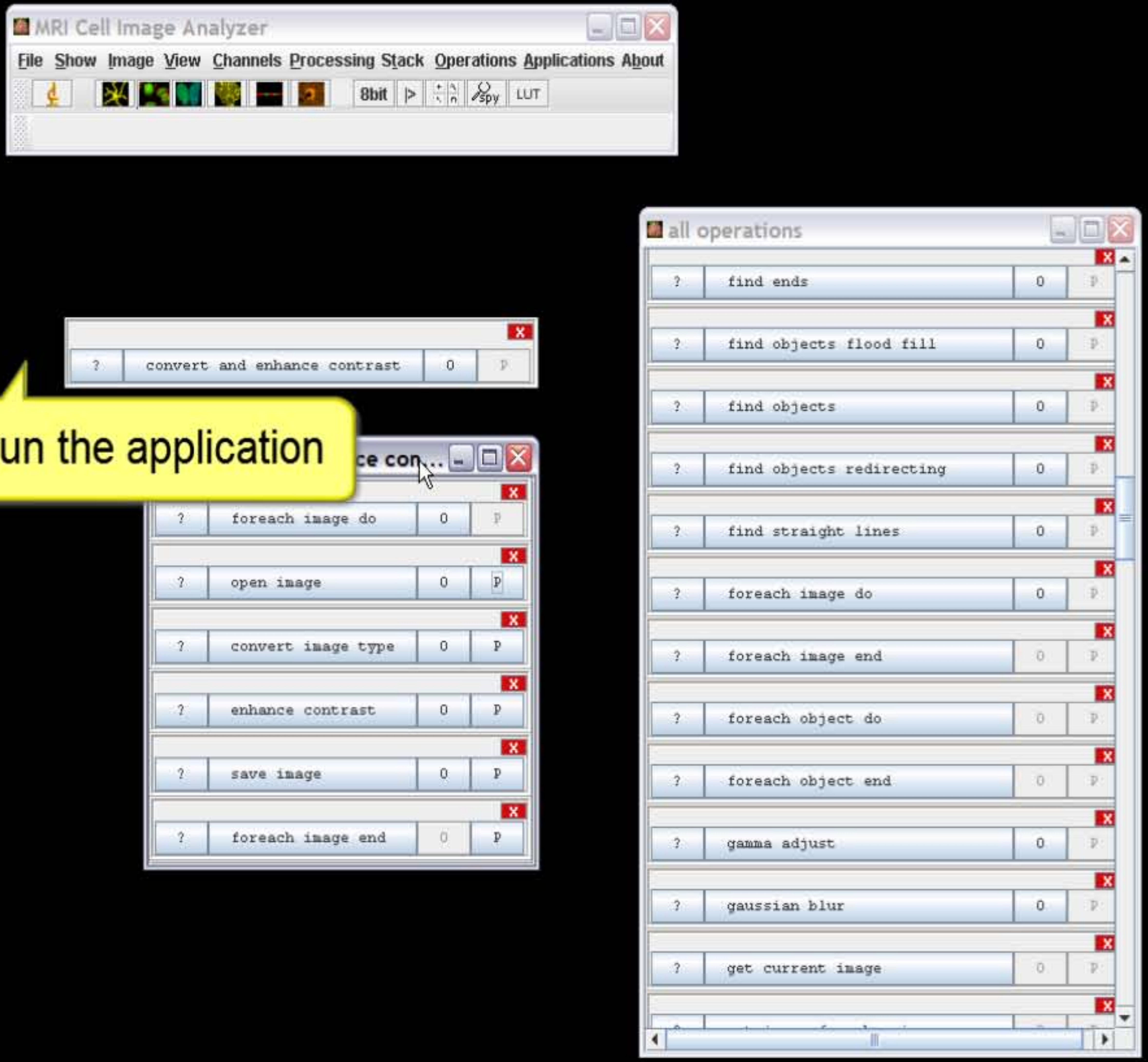
Back Next



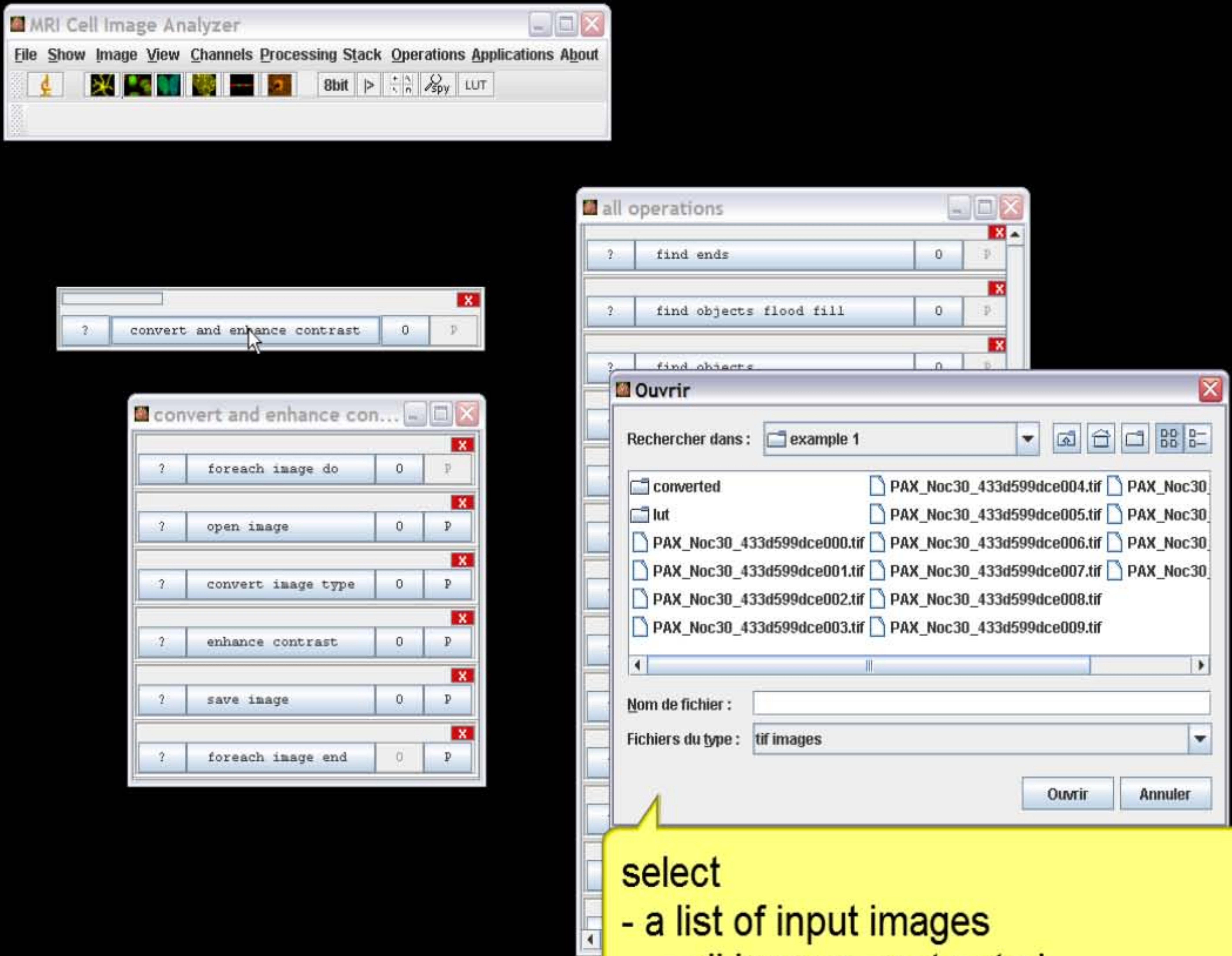
parameters for open image

(String) path := foreach image do : CurrentFilename

parameter	from operation	output
path	foreach image do	current filename



run the application


MRI Cell Image Analyzer
File Show Image View Channels Processing Stack Operations Applications About
8bit
LUT
all operations
Ouvrir
convert and enhance con...

?	find ends	0	P
?	find objects flood fill	0	P
?	find objects	0	P

?	convert and enhance contrast	0	P

?	foreach image do	0	P
?	open image	0	P
?	convert image type	0	P
?	enhance contrast	0	P
?	save image	0	P
?	foreach image end	0	P

Rechercher dans : example 1

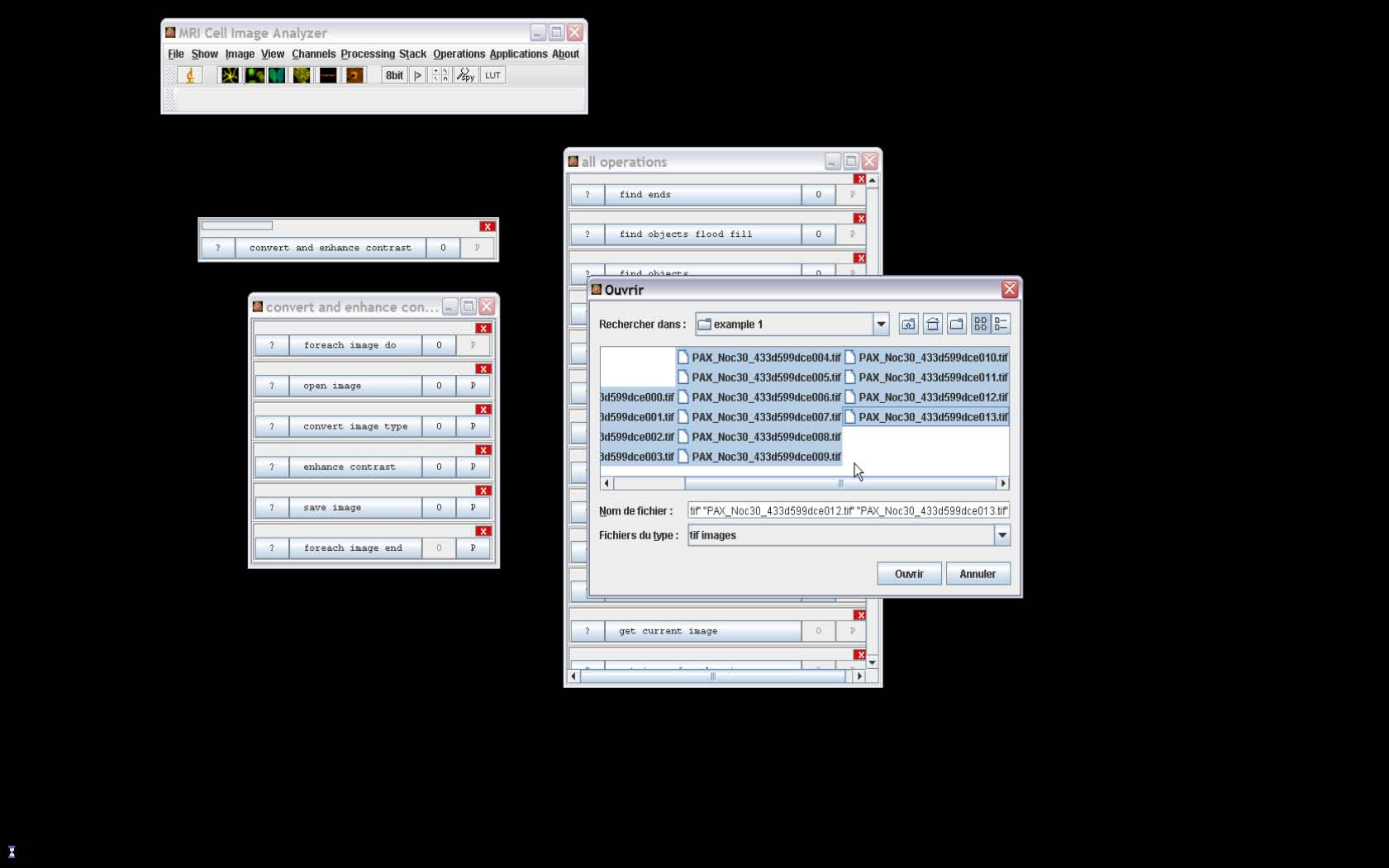
converted
lut
PAX_Noc30_433d599dce000.tif
PAX_Noc30_433d599dce001.tif
PAX_Noc30_433d599dce002.tif
PAX_Noc30_433d599dce003.tif
PAX_Noc30_433d599dce004.tif
PAX_Noc30_433d599dce005.tif
PAX_Noc30_433d599dce006.tif
PAX_Noc30_433d599dce007.tif
PAX_Noc30_433d599dce008.tif
PAX_Noc30_433d599dce009.tif
PAX_Noc30_
PAX_Noc30_
PAX_Noc30_
PAX_Noc30_

Nom de fichier :

Fichiers du type : tif images

select
- a list of input images
  - all images are treated
- one folder
  - all images in the folder and in all sub-folders are treated

Back Next

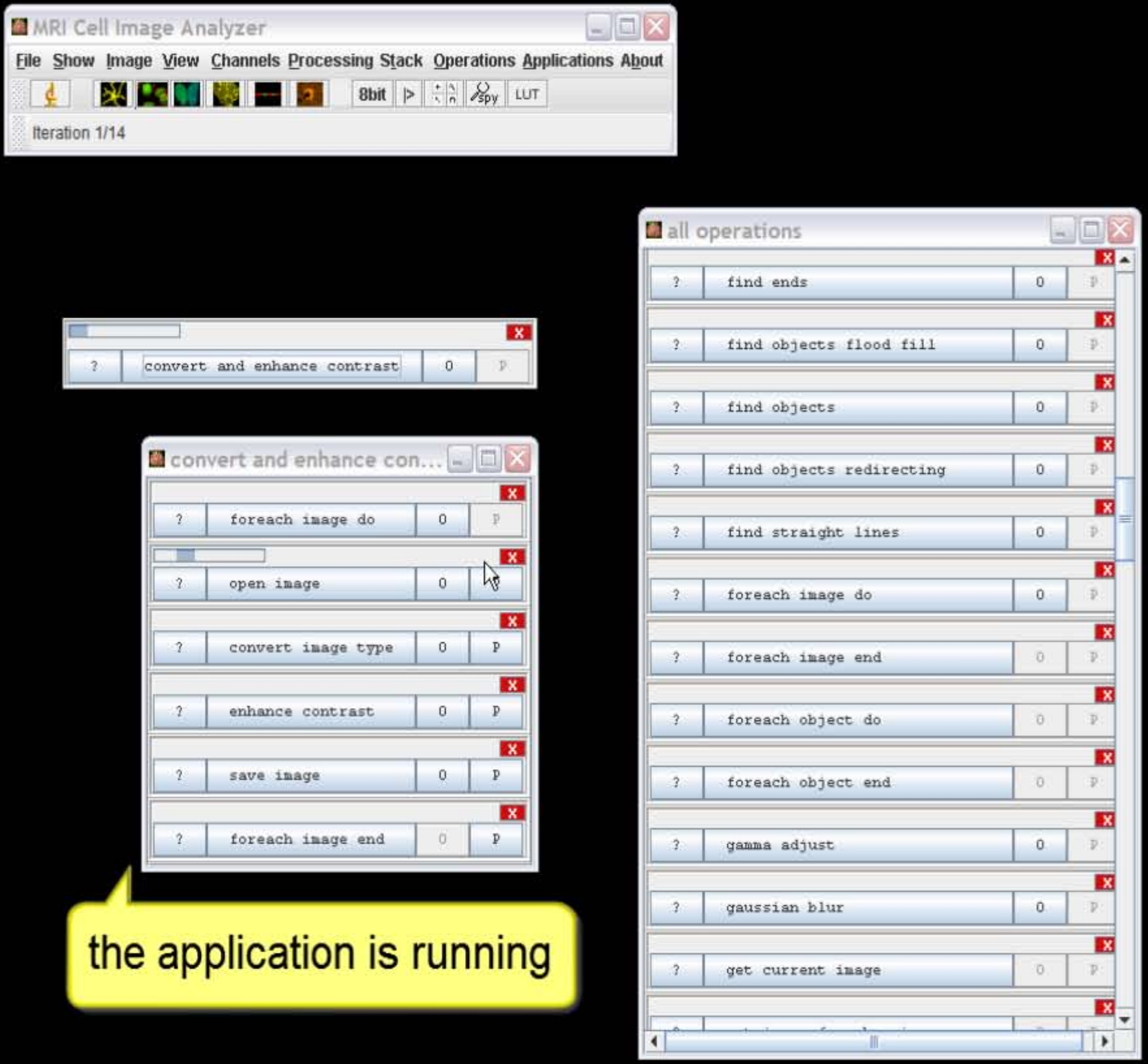


Rechercher dans : example 1

PAX_Noc30_433d599dce004.tif
PAX_Noc30_433d599dce005.tif
3d599dce000.tif
PAX_Noc30_433d599dce006.tif
3d599dce001.tif
PAX_Noc30_433d599dce007.tif
3d599dce002.tif
PAX_Noc30_433d599dce008.tif
3d599dce003.tif
PAX_Noc30_433d599dce009.tif
PAX_Noc30_433d599dce010.tif
PAX_Noc30_433d599dce011.tif
PAX_Noc30_433d599dce012.tif
PAX_Noc30_433d599dce013.tif

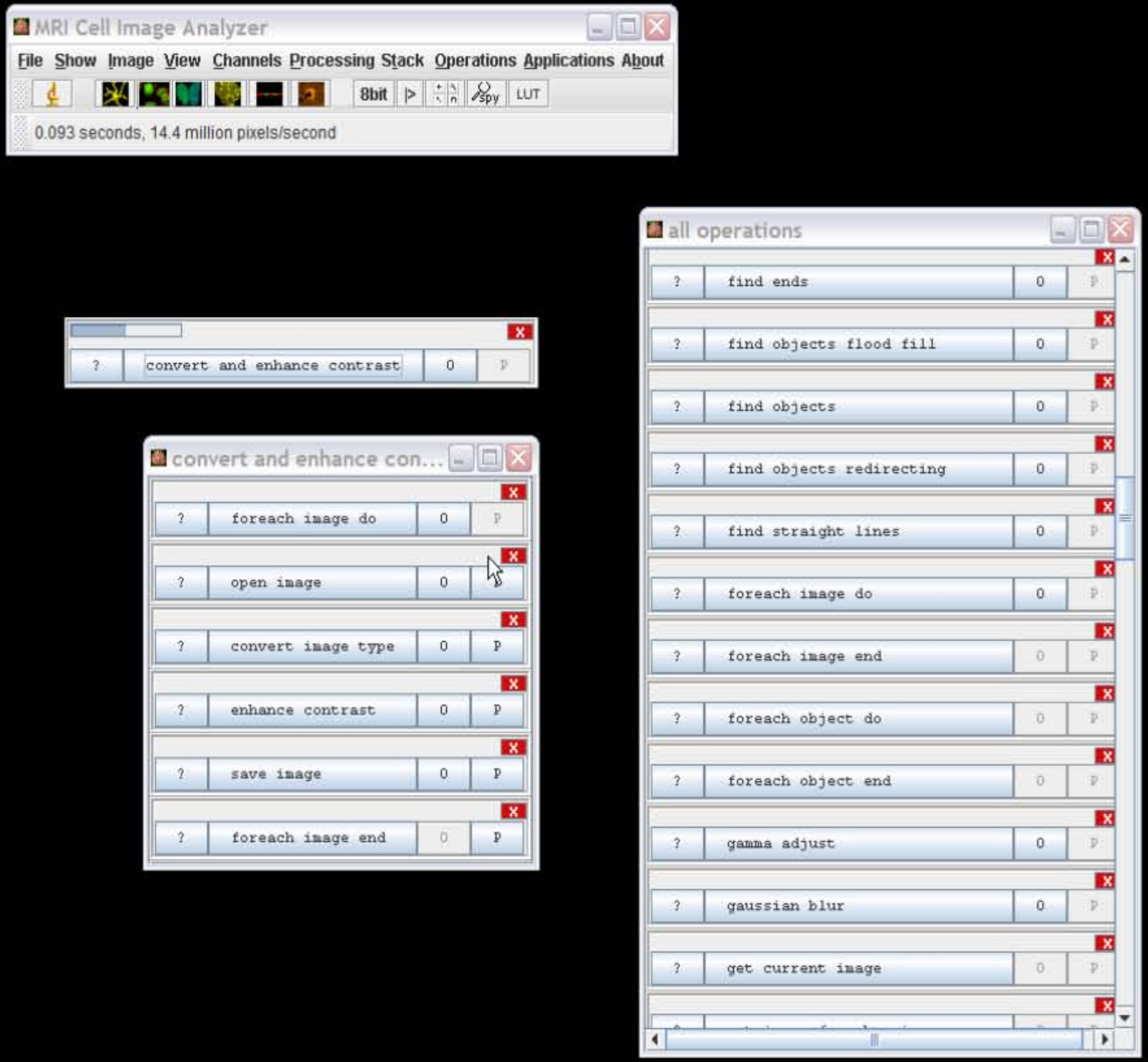
Nom de fichier : tif" "PAX_Noc30_433d599dce012.tif" "PAX_Noc30_433d599dce013.tif"

Fichiers du type : tif images

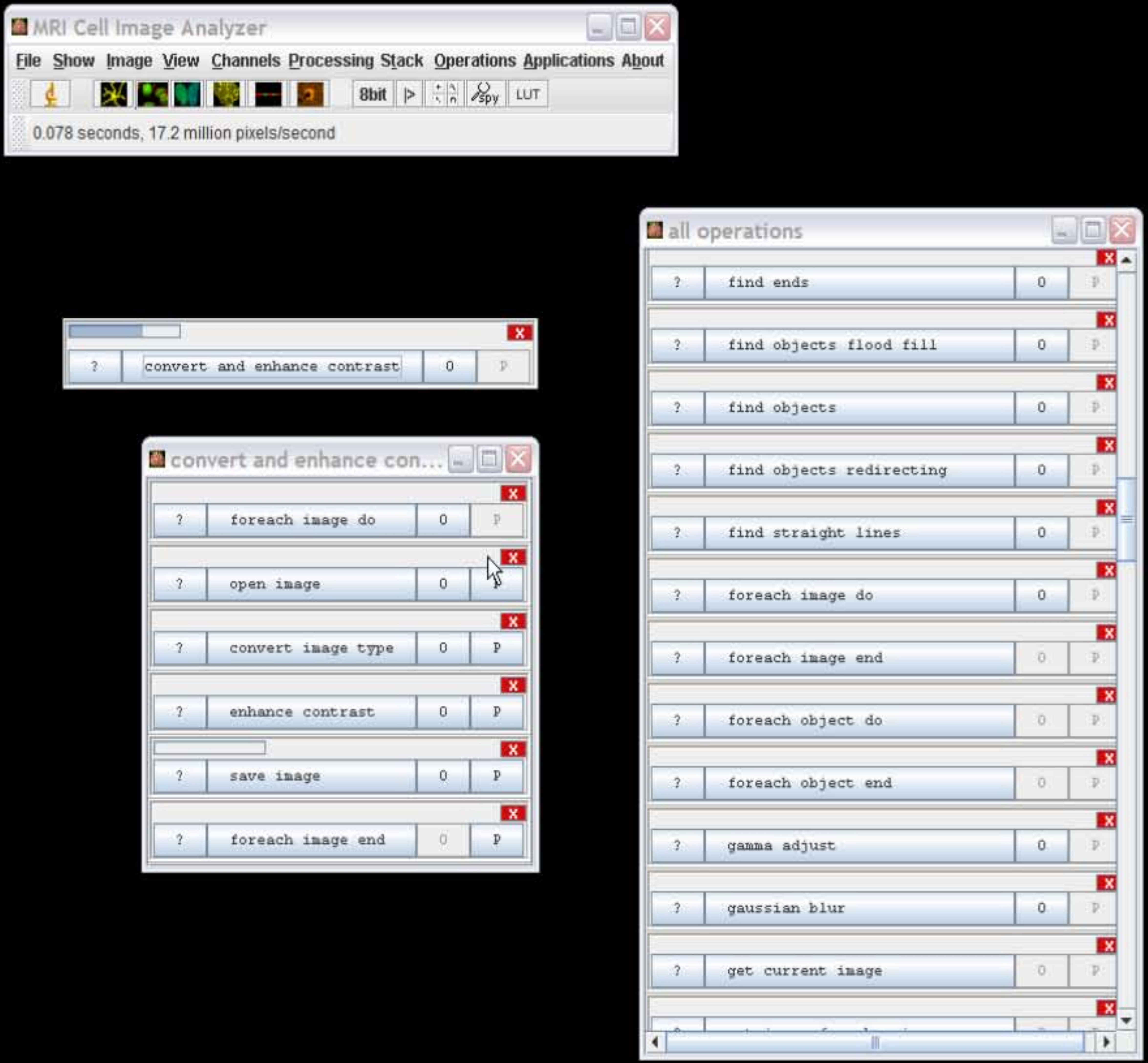


the application is running

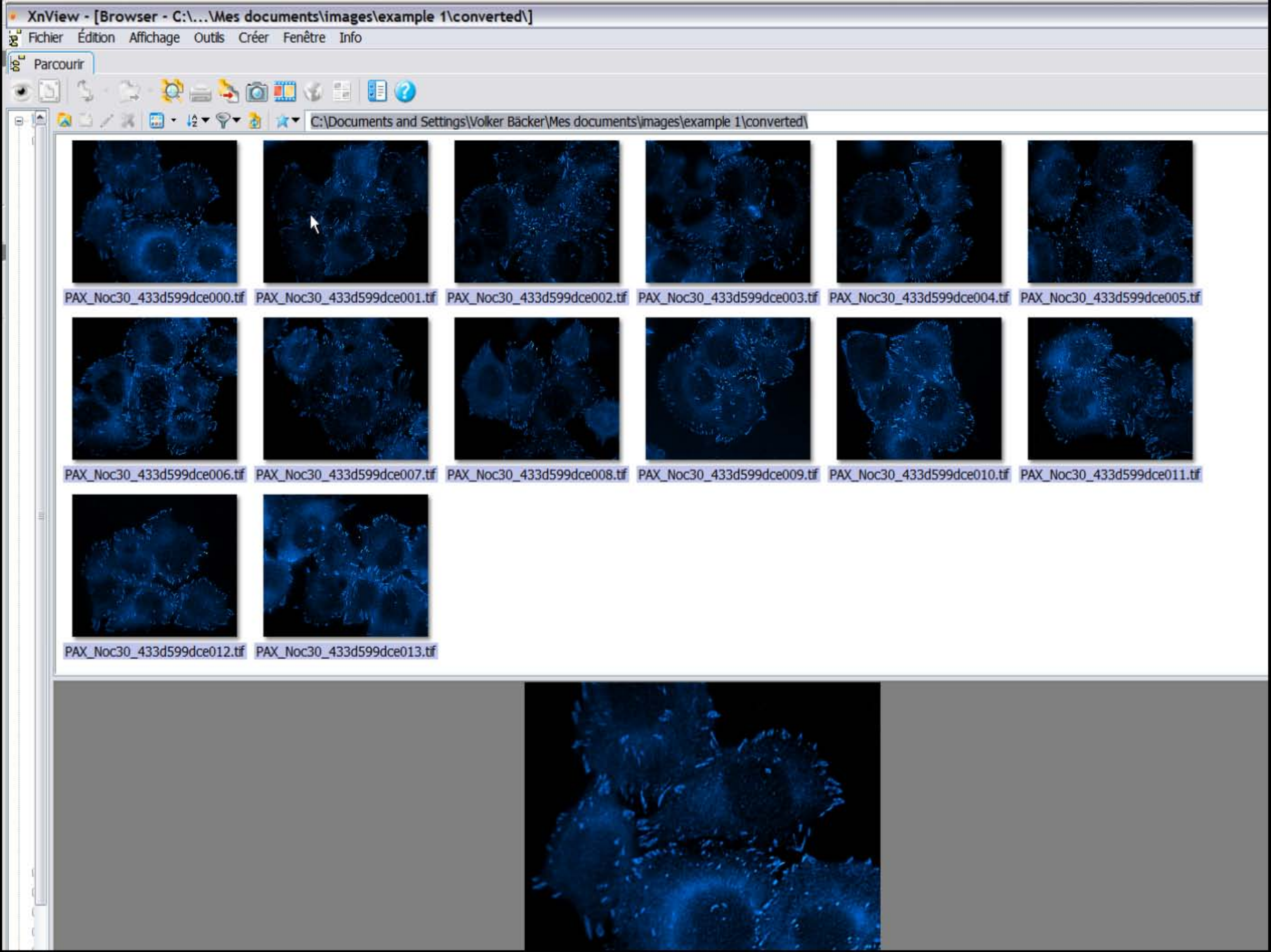
Back Next



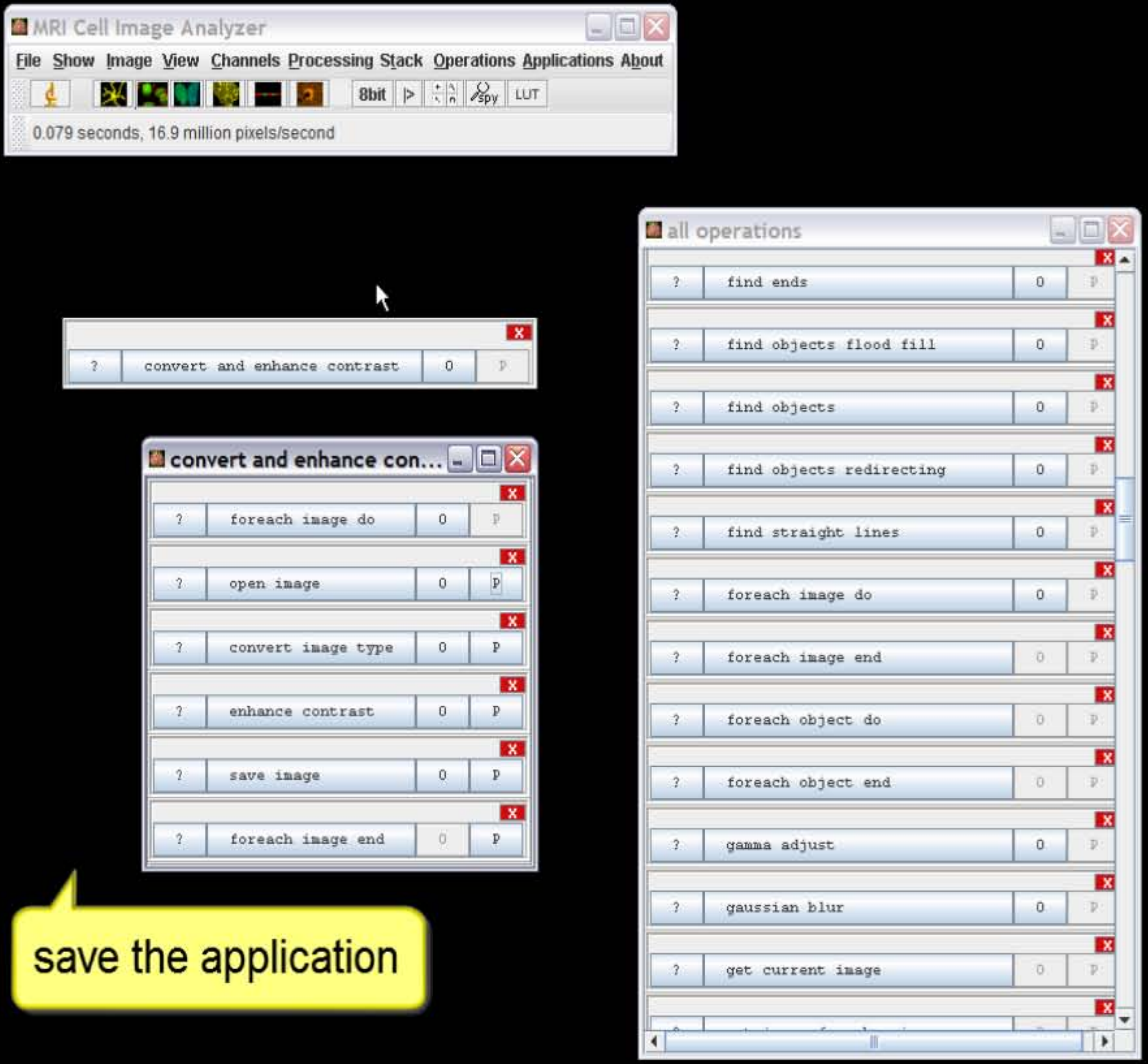
0.093 seconds, 14.4 million pixels/second



0.078 seconds, 17.2 million pixels/second


XnView - [Browser - C:\...\Mes documents\images\example 1\converted\]
Fichier
Édition
Affichage
Outils
Créer
Fenêtre
Info
Parcourir
PAX_Noc30_433d599dce000.tif
PAX_Noc30_433d599dce001.tif
PAX_Noc30_433d599dce002.tif
PAX_Noc30_433d599dce003.tif
PAX_Noc30_433d599dce004.tif
PAX_Noc30_433d599dce005.tif
PAX_Noc30_433d599dce006.tif
PAX_Noc30_433d599dce007.tif
PAX_Noc30_433d599dce008.tif
PAX_Noc30_433d599dce009.tif
PAX_Noc30_433d599dce010.tif
PAX_Noc30_433d599dce011.tif
PAX_Noc30_433d599dce012.tif
PAX_Noc30_433d599dce013.tif

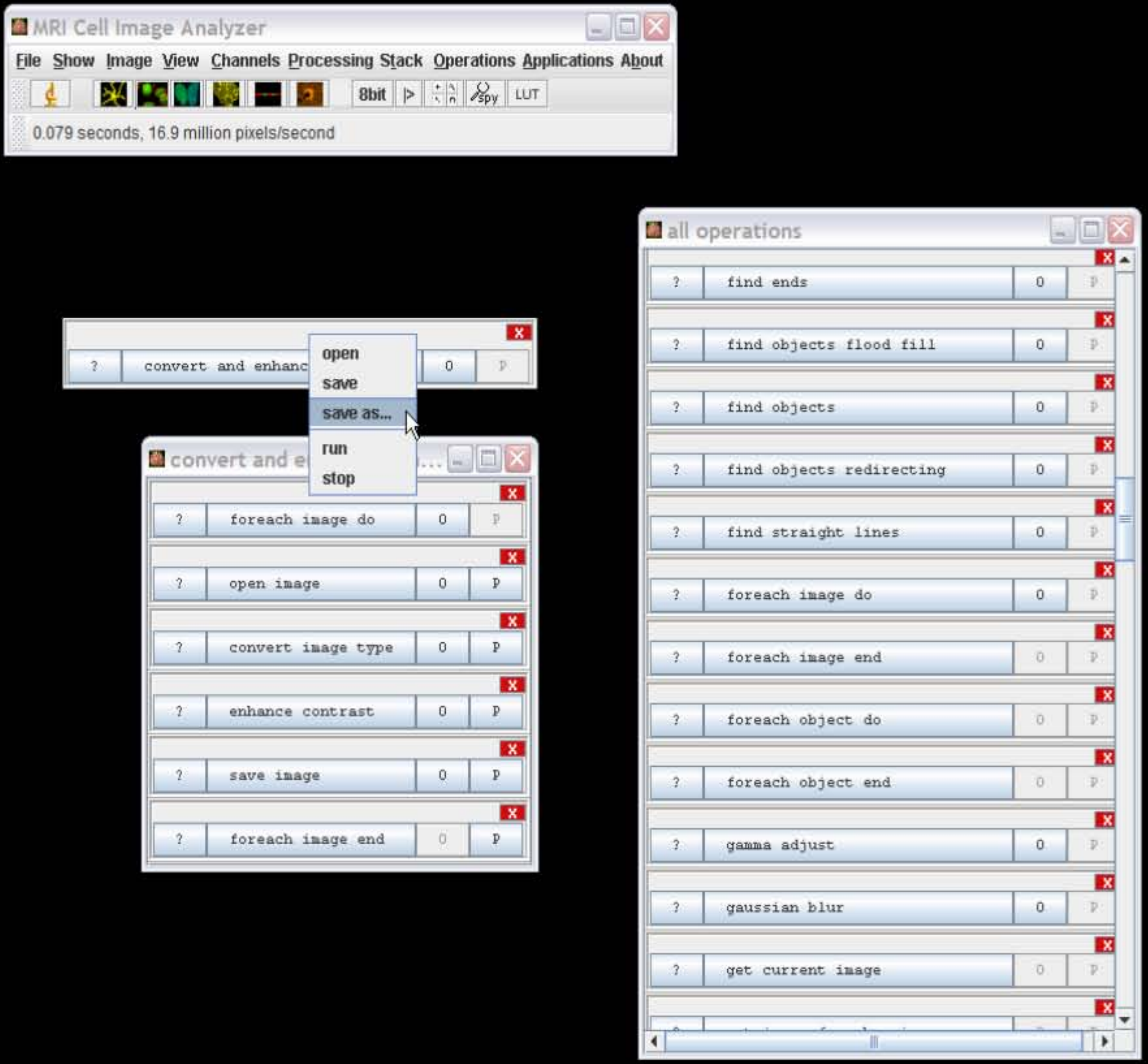
C:\Documents and Settings\Volker Bäcker\Mes documents\images\example 1\converted\



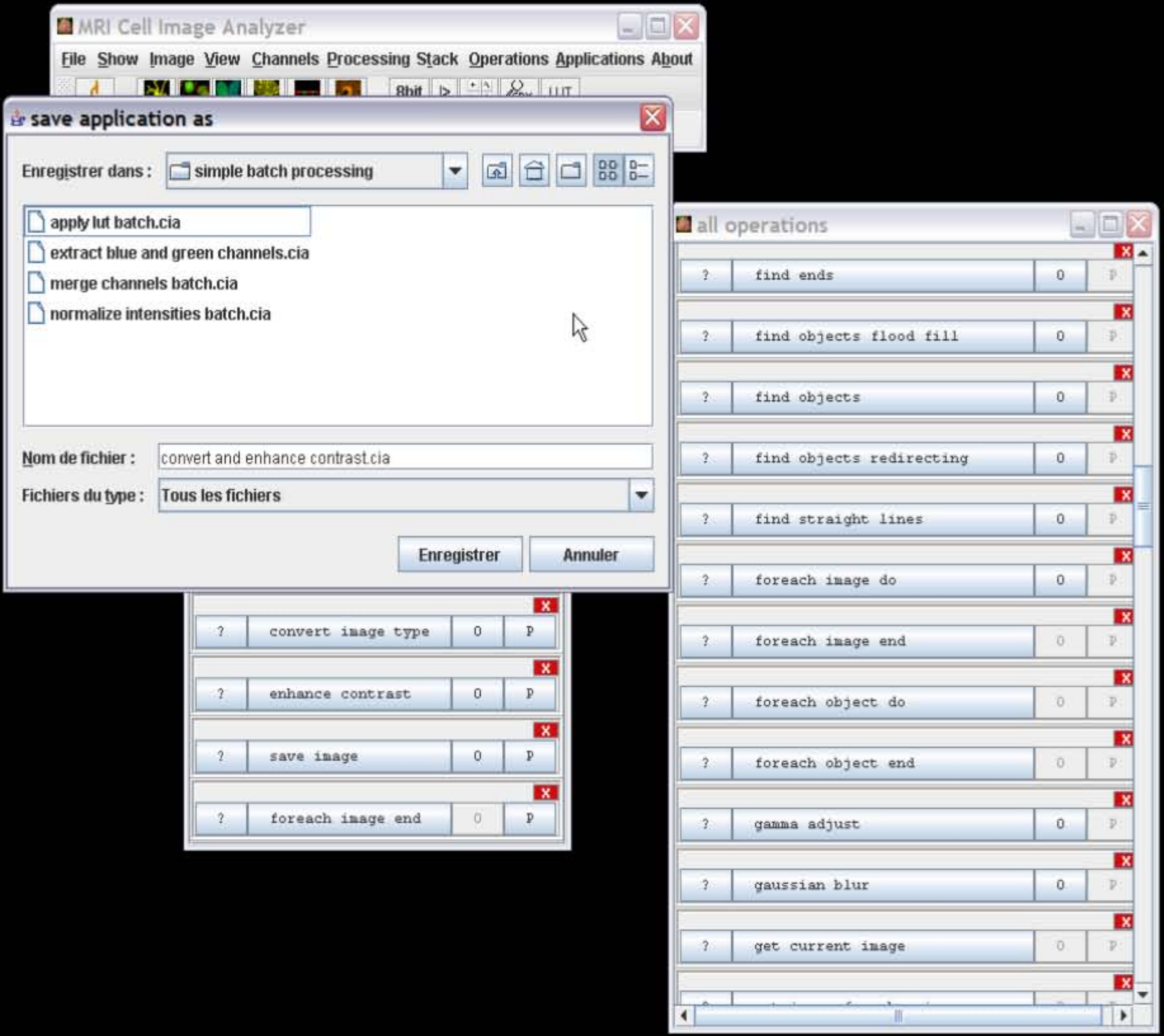
0.079 seconds, 16.9 million pixels/second

save the application

Back



0.079 seconds, 16.9 million pixels/second



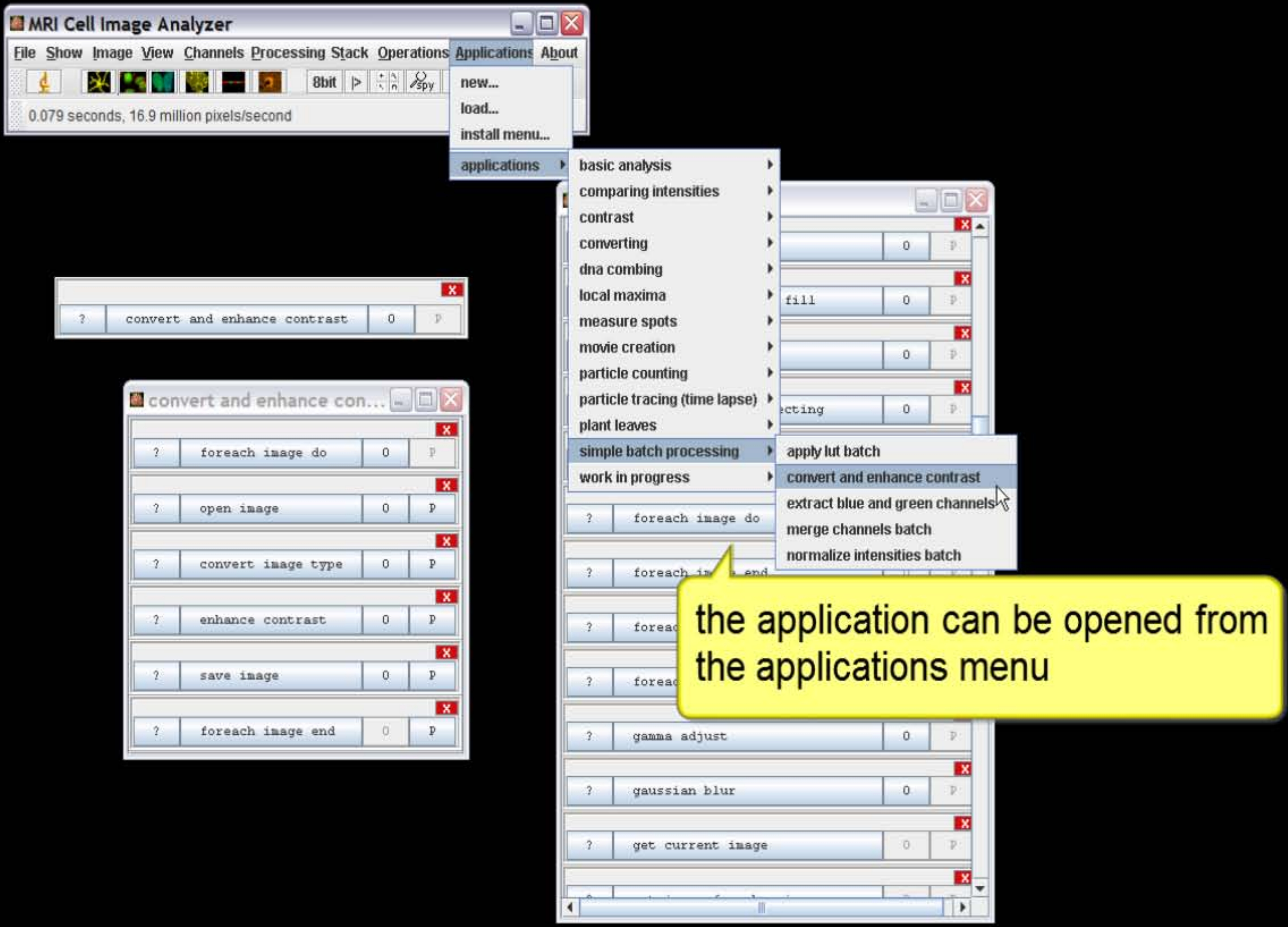
MRI Cell Image Analyzer
File Show Image View Channels Processing Stack Operations Applications About
8bit
LUT
save application as

Enregistrer dans : simple batch processing

apply lut batch.cia
extract blue and green channels.cia
merge channels batch.cia
normalize intensities batch.cia

Nom de fichier : convert and enhance contrast.cia

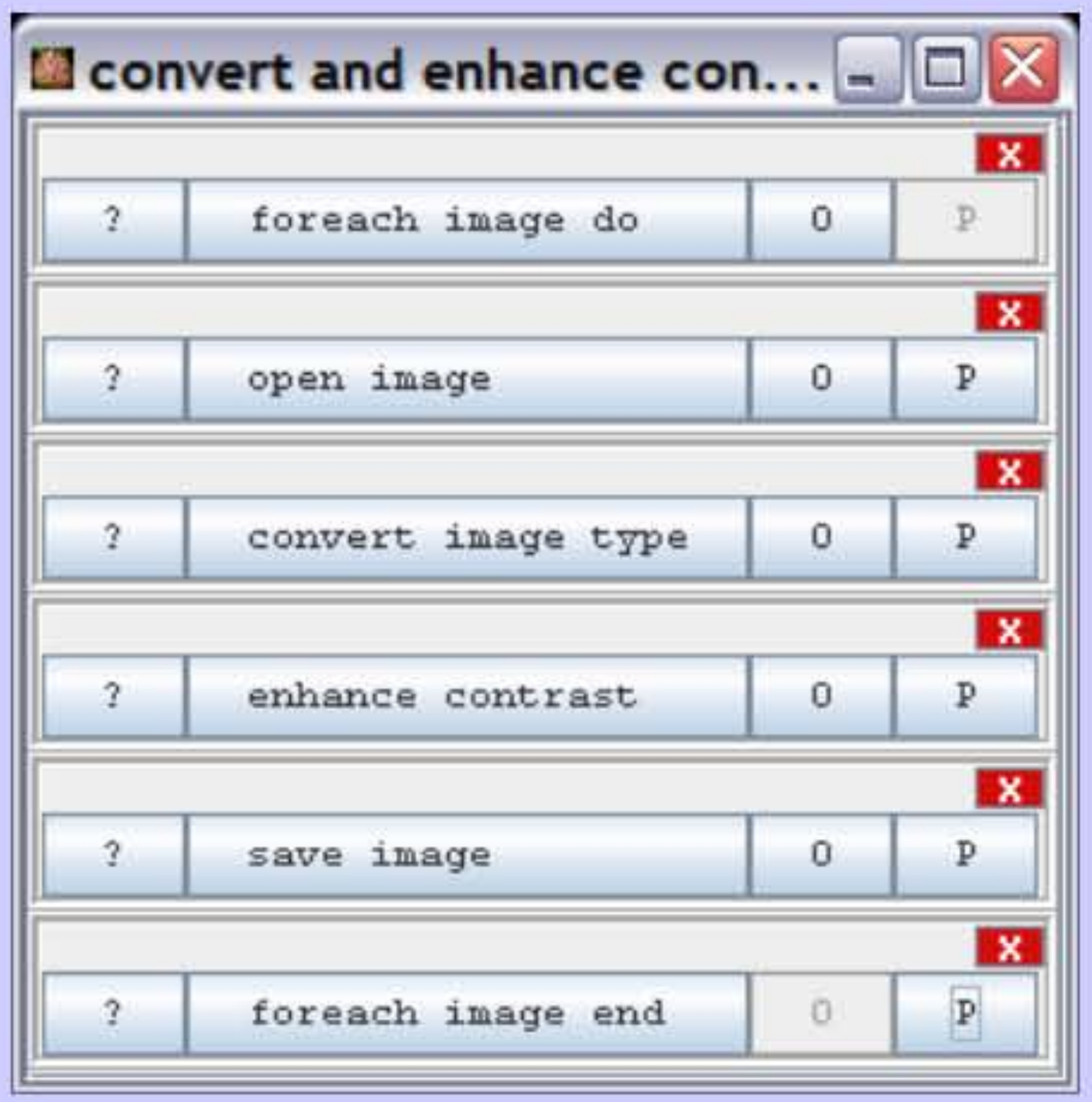
Fichiers du type : Tous les fichiers



the application can be opened from the applications menu

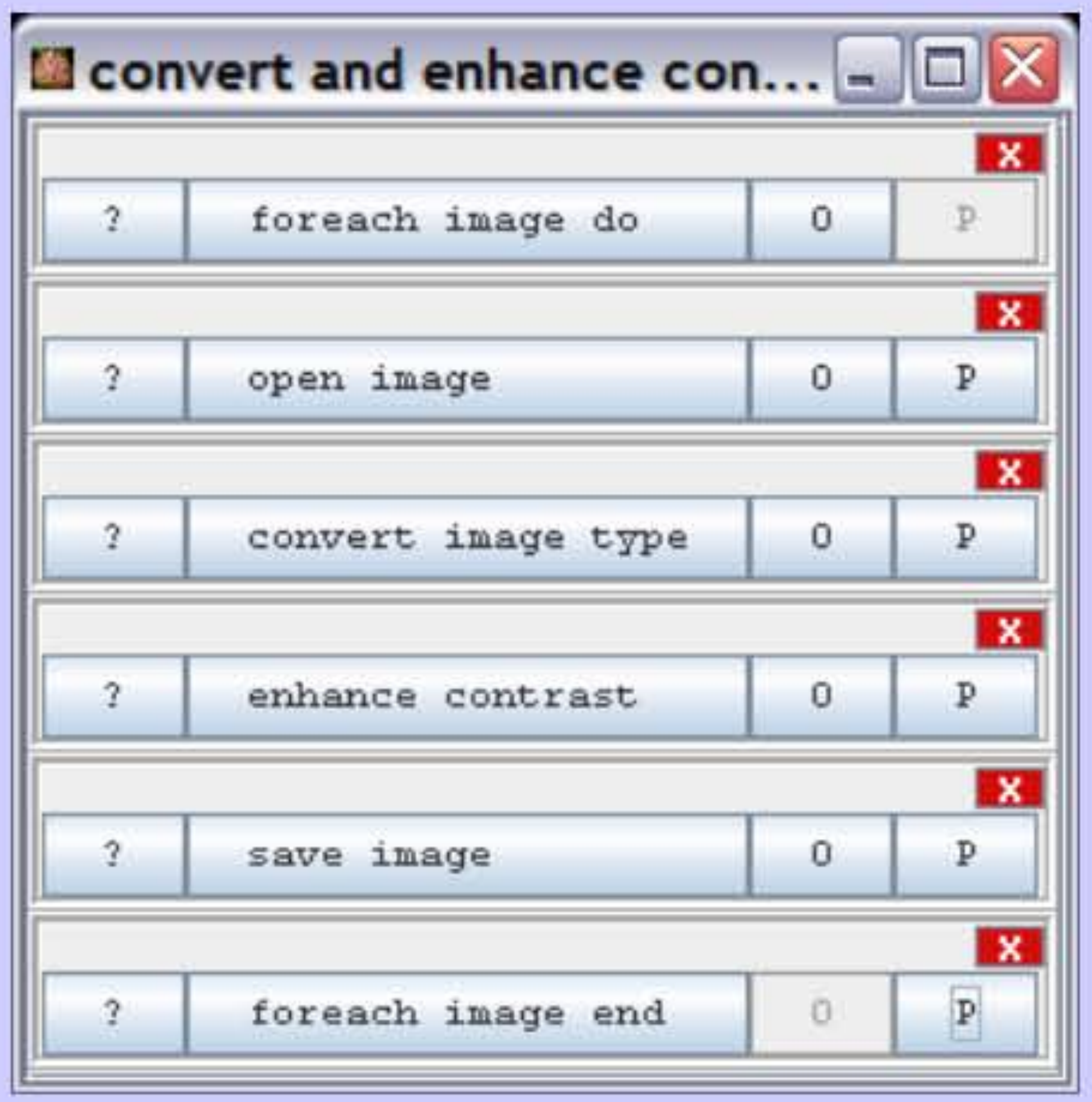
Back

Comparison visual script / macro



```
dir1 = getDirectory("Choose Source Directory ");
dir2 = getDirectory("Choose Destination Directory ");
list = getFileList(dir1);
Dialog.create("enhance contrast options");
Dialog.addNumber("percent saturated", 0.5);
Dialog.addCheckbox("equalize", false);
Dialog.addCheckbox("normalize", true);
Dialog.addCheckbox("use stack histogram", false);
Dialog.show();
saturated=Dialog.getNumber();
equalize = Dialog.getCheckbox();
normalize = Dialog.getCheckbox();
useStackHistogram = equalize = Dialog.getCheckbox();
setBatchMode(true);
for (i=0; i<list.length; i++) {
   showProgress(i+1, list.length);
   if (endsWith(list[i], ".tif")) {
      open(dir1+list[i]);
      run("8-bit");
      run("Enhance Contrast", "saturated=" + saturated + "
     normalize");
      saveAs("Tiff",  dir2+list[i]);
   }
}
```

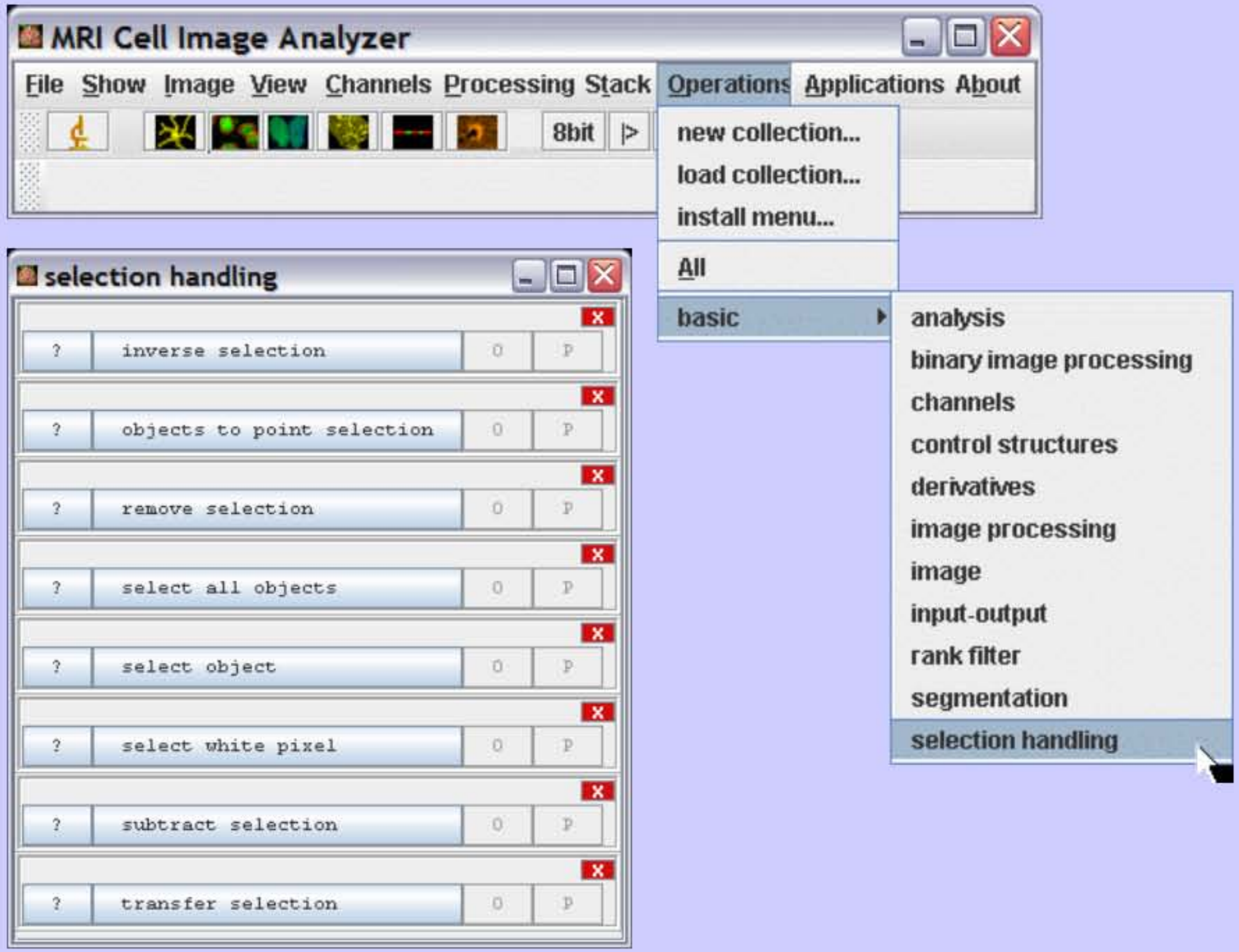
Comparison visual script / macro



```
dir1 = getDirectory("Choose Source Directory ");
dir2 = getDirectory("Choose Destination Directory ");
list = getFileList(dir1);
setBatchMode(true);
for (i=0; i<list.length; i++) {
   showProgress(i+1, list.length);
   if (endsWith(list[i], ".tif")) {
      open(dir1+list[i]);
      run("8-bit");
      run("Enhance Contrast", "saturated=" + saturated + "
     normalize");
      saveAs("Tiff",  dir2+list[i]);
   }
}
```

Operation collections

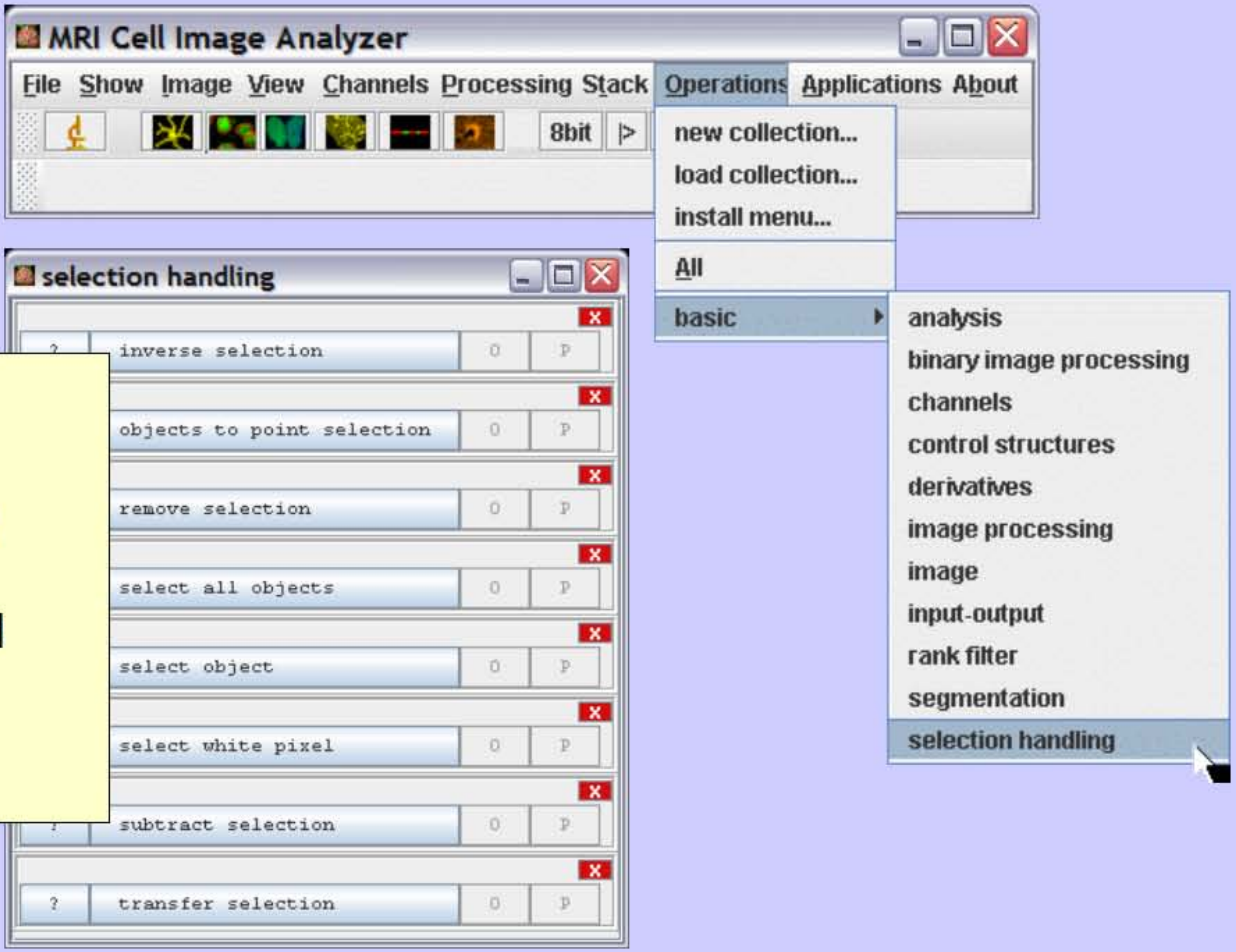
- processing
- analysis
- control structures
- selection handling
- input / output
- reporting



Operation collections

- processing
- analysis
- control structures

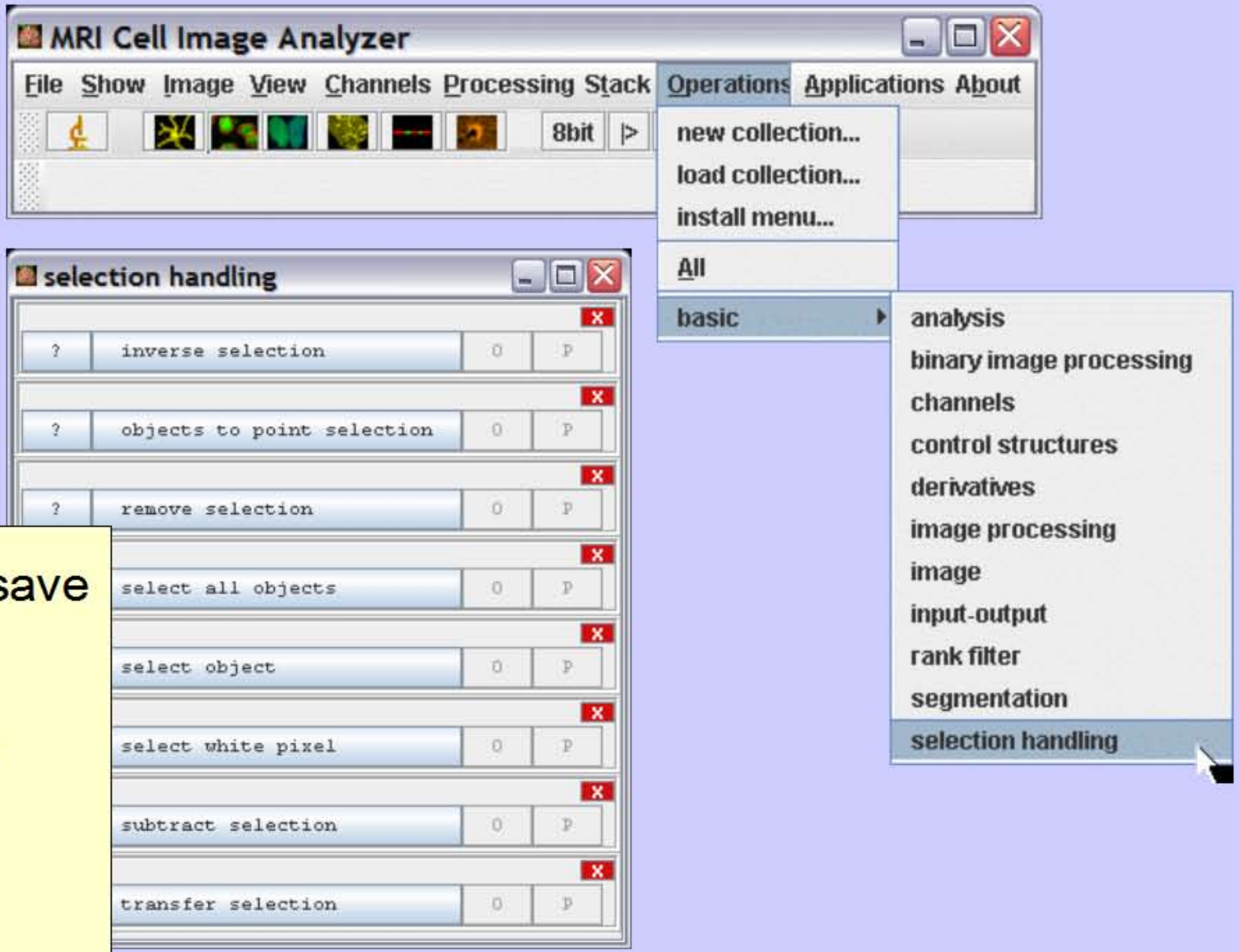
foreach image do
foreach object do
accept or skip or exit
wait for user
skip no objects found
skip saturated
...



Operation collections

- processing
- analysis
- control structures
- selection handling
- input / output

open / show / hide / save / close image
show results table
open series as stack
save as tiff series



MRI Cell Image Analyzer

File Show Image View Channels Processing Stack Operations Applications About

8bit

new collection...
load collection...
install menu...
All
basic

analysis
binary image processing
channels
control structures
derivatives
image processing
image
input-output
rank filter
segmentation
selection handling

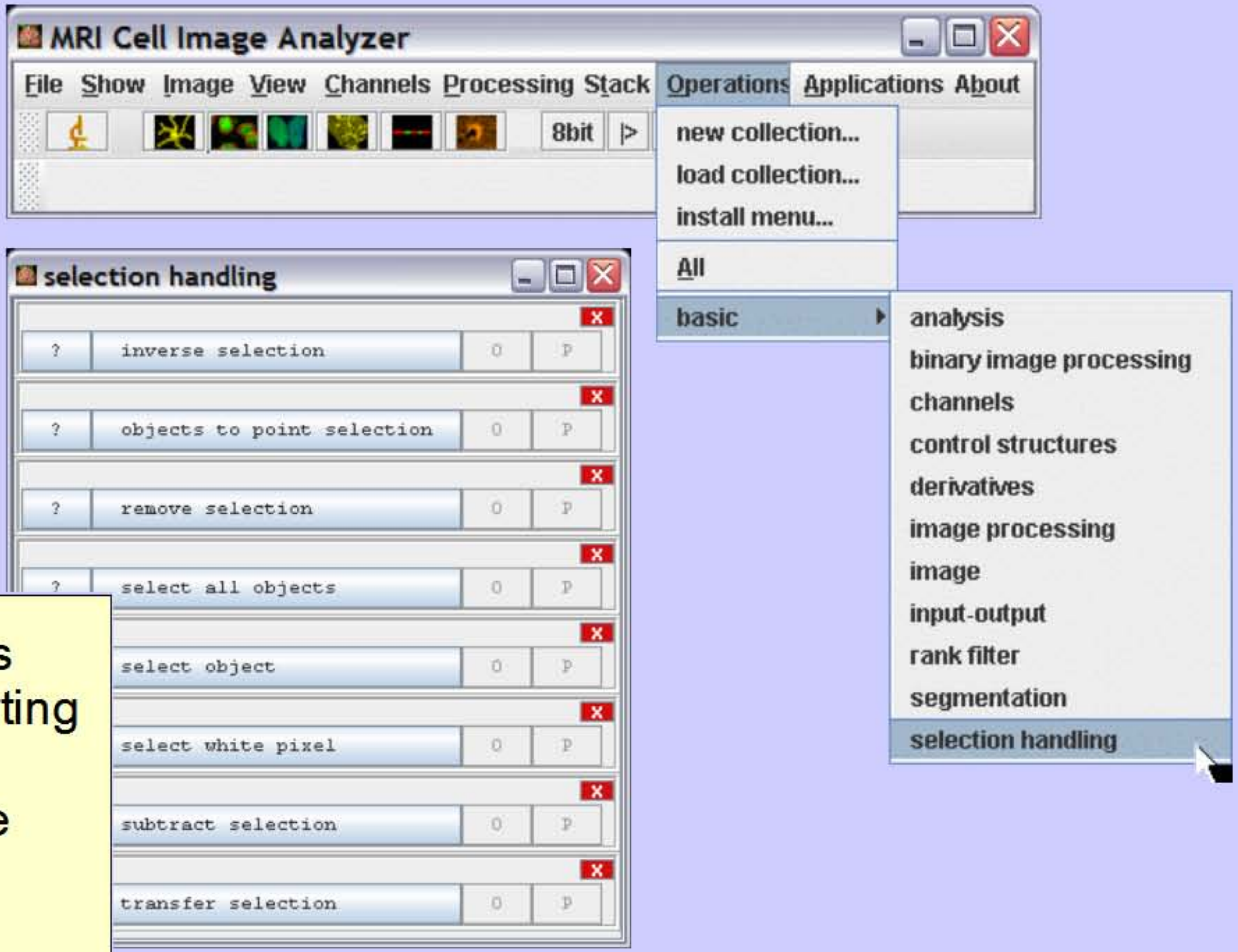
selection handling

?	inverse selection	O	P
?	objects to point selection	O	P
?	remove selection	O	P
	select all objects	O	P
	select object	O	P
	select white pixel	O	P
	subtract selection	O	P
	transfer selection	O	P

Operation collections

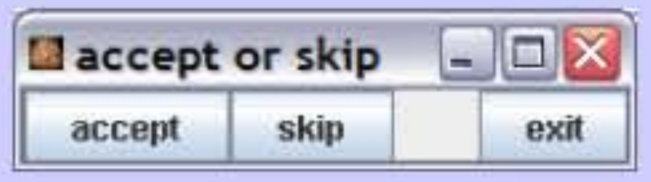
- processing
- analysis
- control structures
- selection handling
- input / output
- reporting

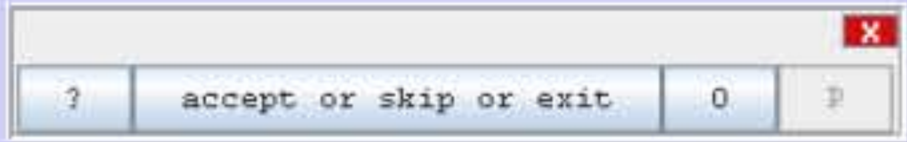
report measurements
project specific reporting
operations
write spreadsheet file

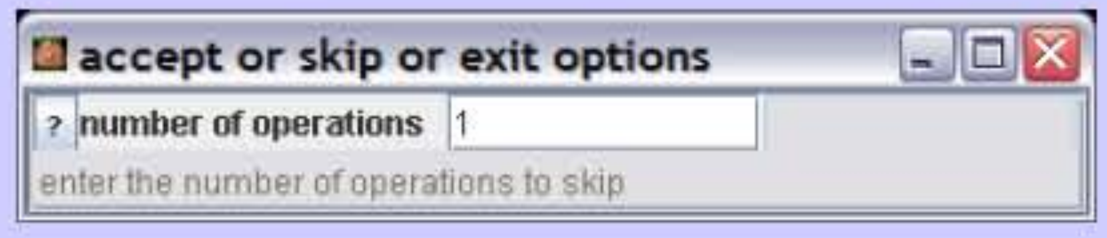


Interactive applications

- let the computer do the work when possible
- the user take decisions when necessary


accept or skip



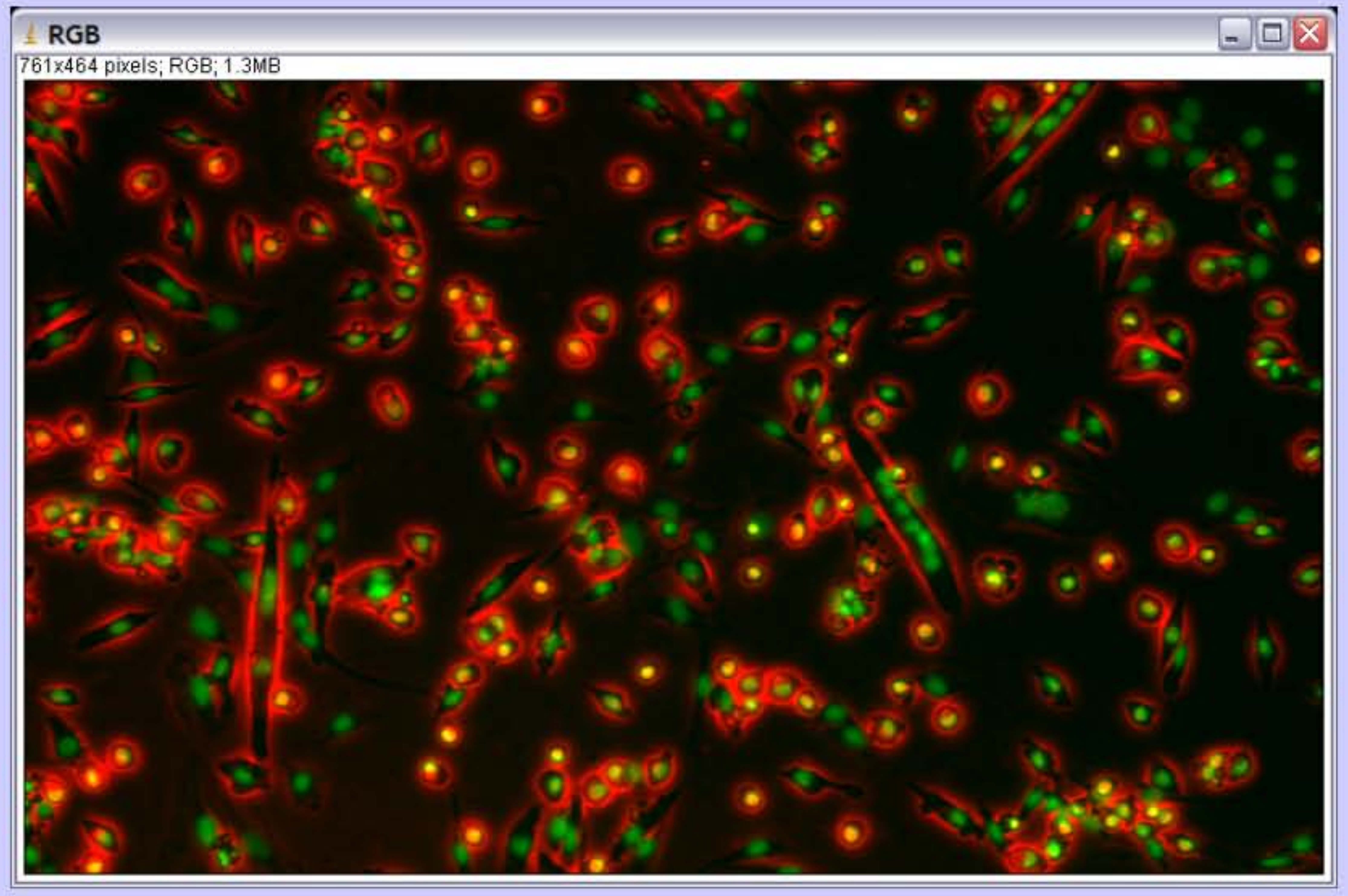

accept or skip or exit options

? number of operations 1

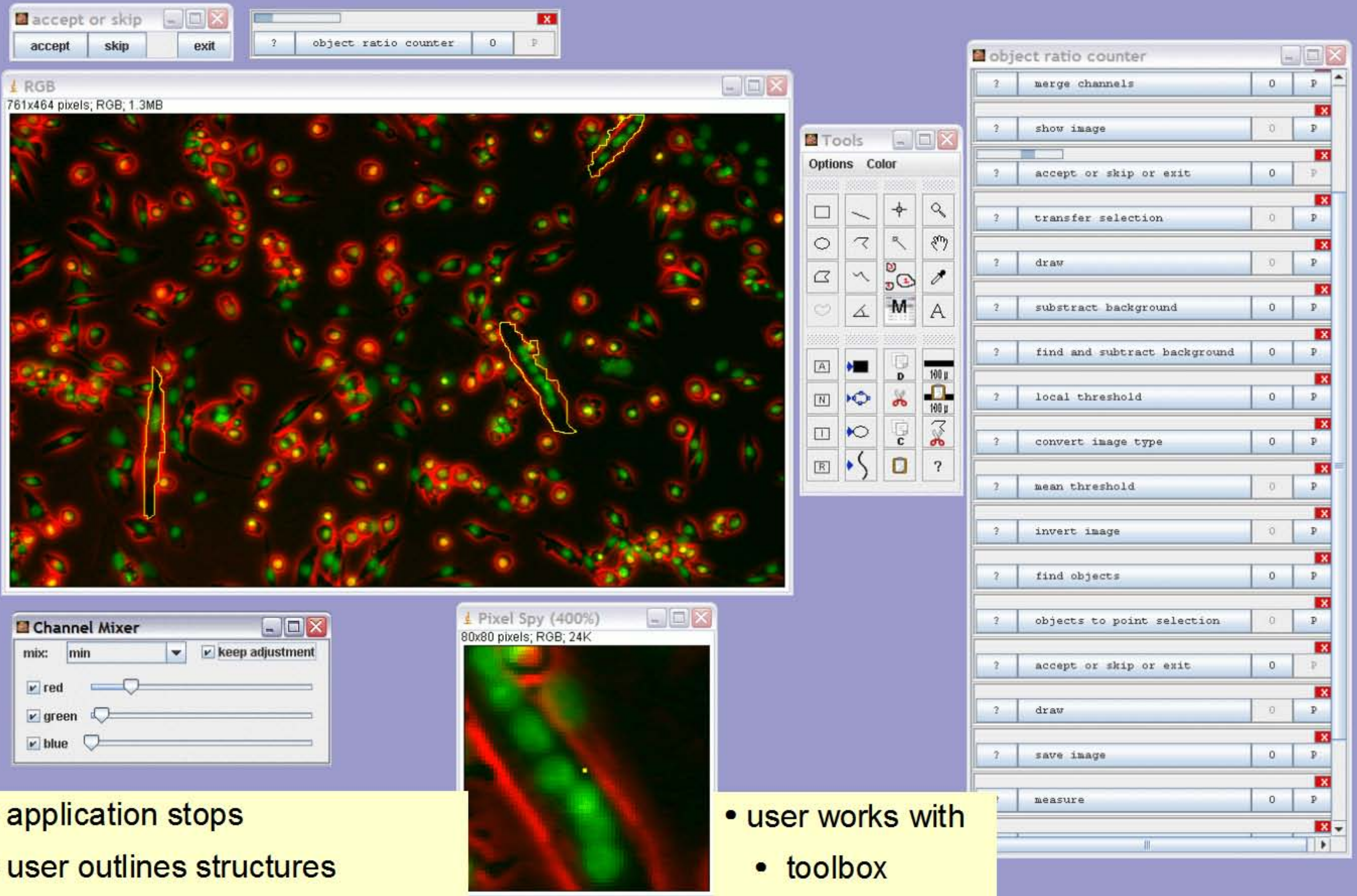
enter the number of operations to skip

- execution of application stops
- user decides to take result into account
- user corrects automatic result
  - selection
  - mask

Interactive applications



- count nuclei
  - all
  - inside certain structures
- calculate the ratio

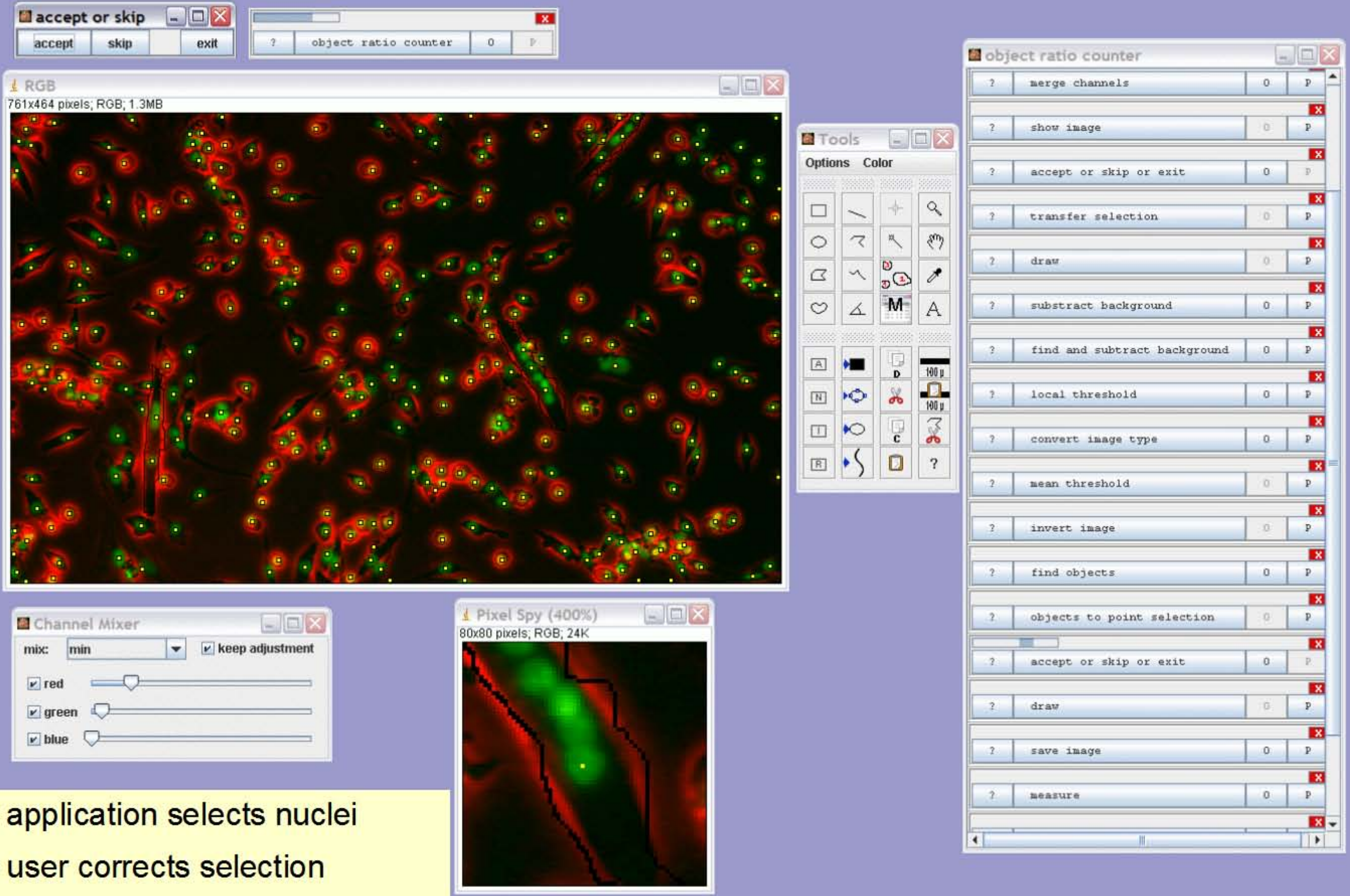


mix: min
☑ keep adjustment
☑ red
☑ green
☑ blue

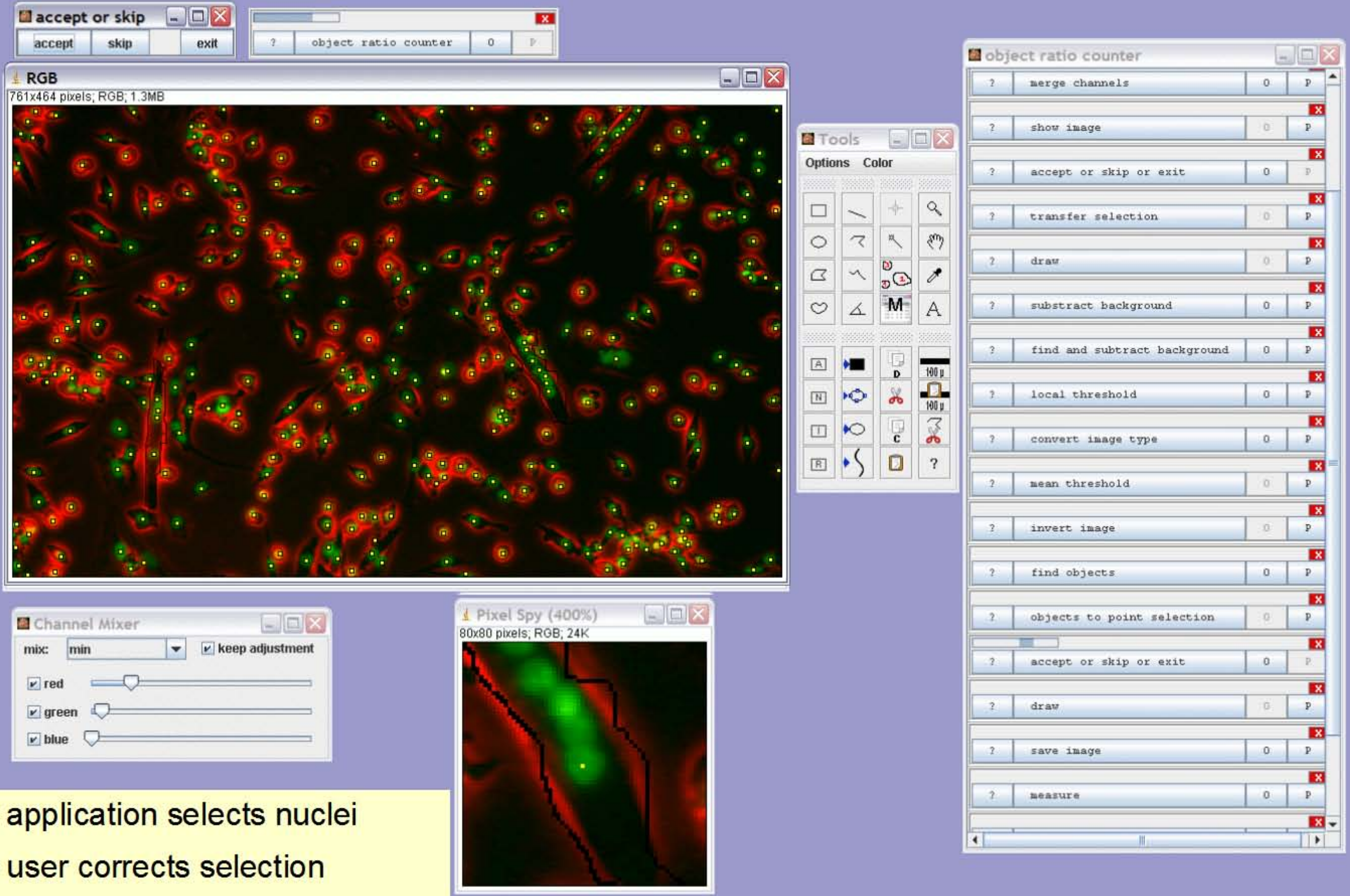
merge channels
show image
accept or skip or exit
transfer selection
draw
substract background
find and subtract background
local threshold
convert image type
mean threshold
invert image
find objects
objects to point selection
accept or skip or exit
draw
save image
measure

- application stops
- user outlines structures
- user presses accept to continue

- user works with
  - toolbox
  - channel mixer
  - pixel spy



- application selects nuclei
- user corrects selection
- user presses accept to continue



RGB
761x464 pixels; RGB; 1.3MB

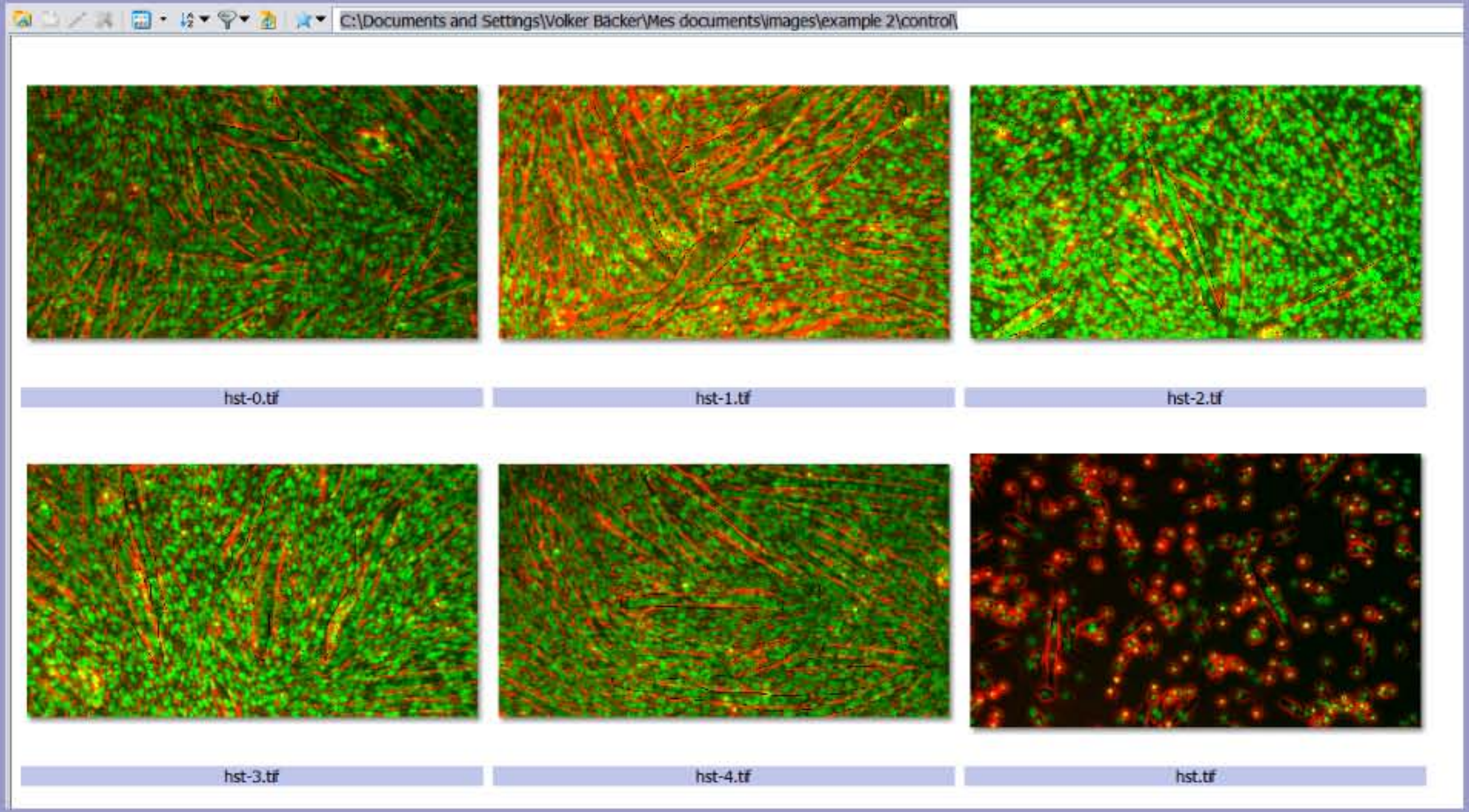
Channel Mixer
mix: min ☑ keep adjustment
☑ red
☑ green
☑ blue

Pixel Spy (400%)
80x80 pixels; RGB; 24K

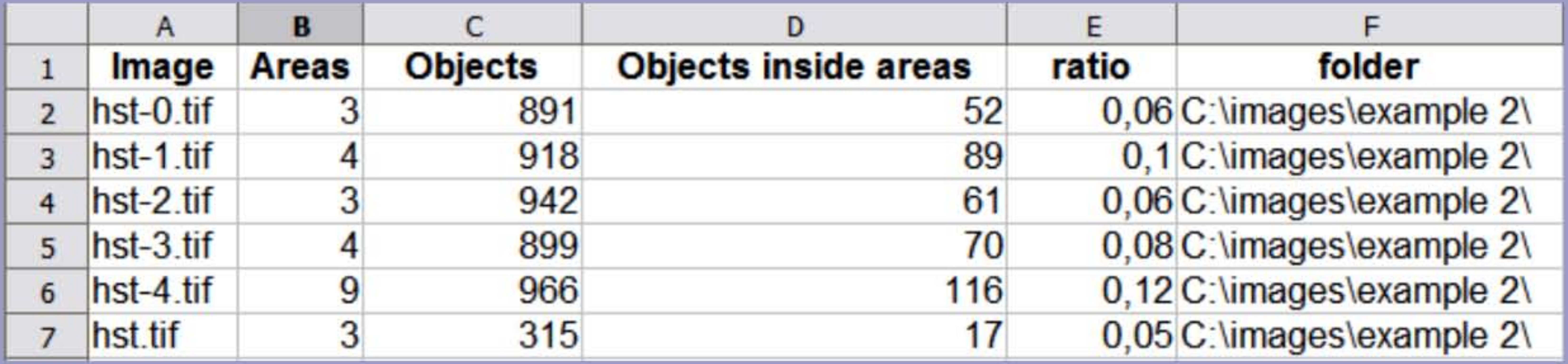
object ratio counter

```
?   merge channels                  0   P
?   show image                      0   P
?   accept or skip or exit          0   P
?   transfer selection              0   P
?   draw                            0   P
?   substract background            0   P
?   find and subtract background    0   P
?   local threshold                 0   P
?   convert image type              0   P
?   mean threshold                  0   P
?   invert image                    0   P
?   find objects                    0   P
?   objects to point selection      0   P
?   accept or skip or exit          0   P
?   draw                            0   P
?   save image                      0   P
?   measure                         0   P
```

- application selects nuclei
- user corrects selection
- user presses accept to continue

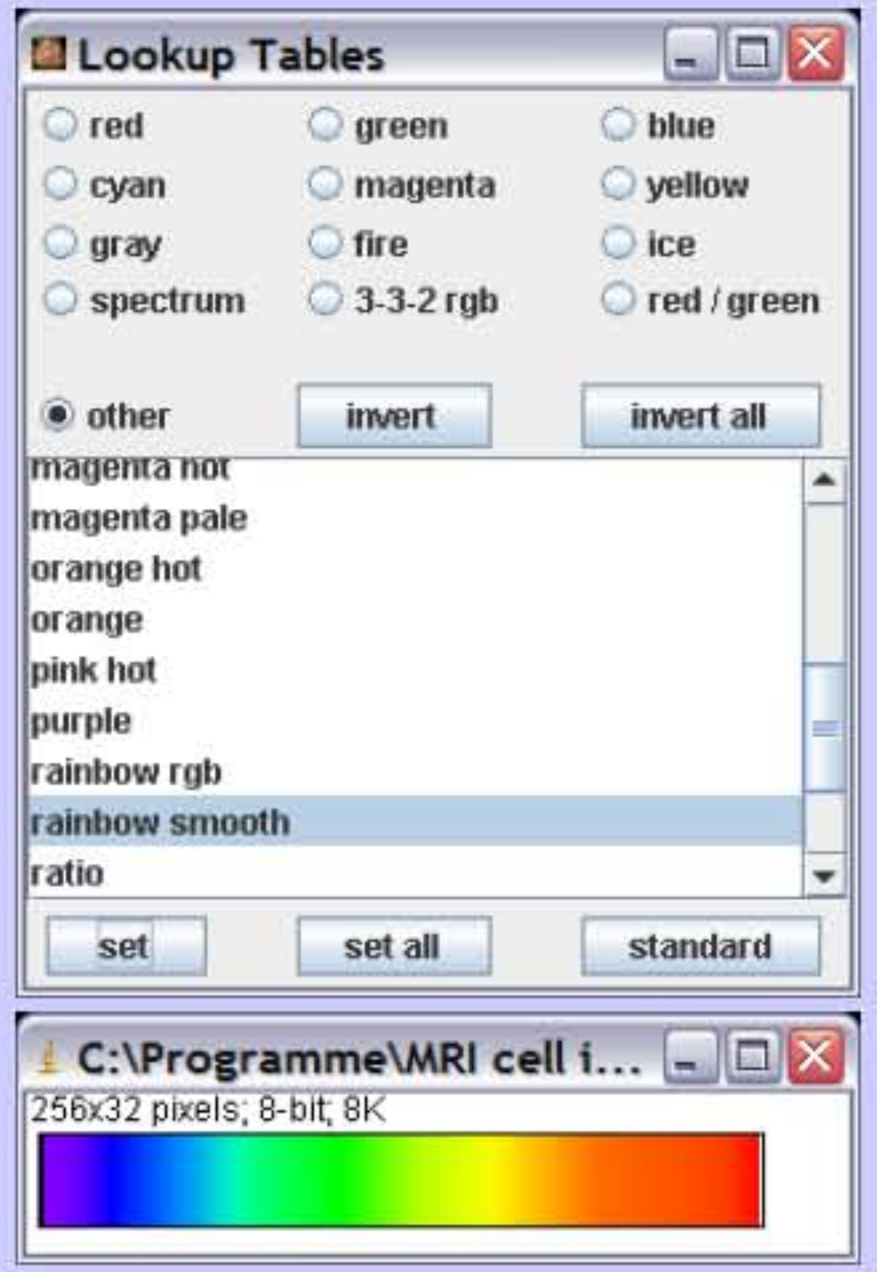


C:\Documents and Settings\Volker Bäcker\Mes documents\images\example 2\control\

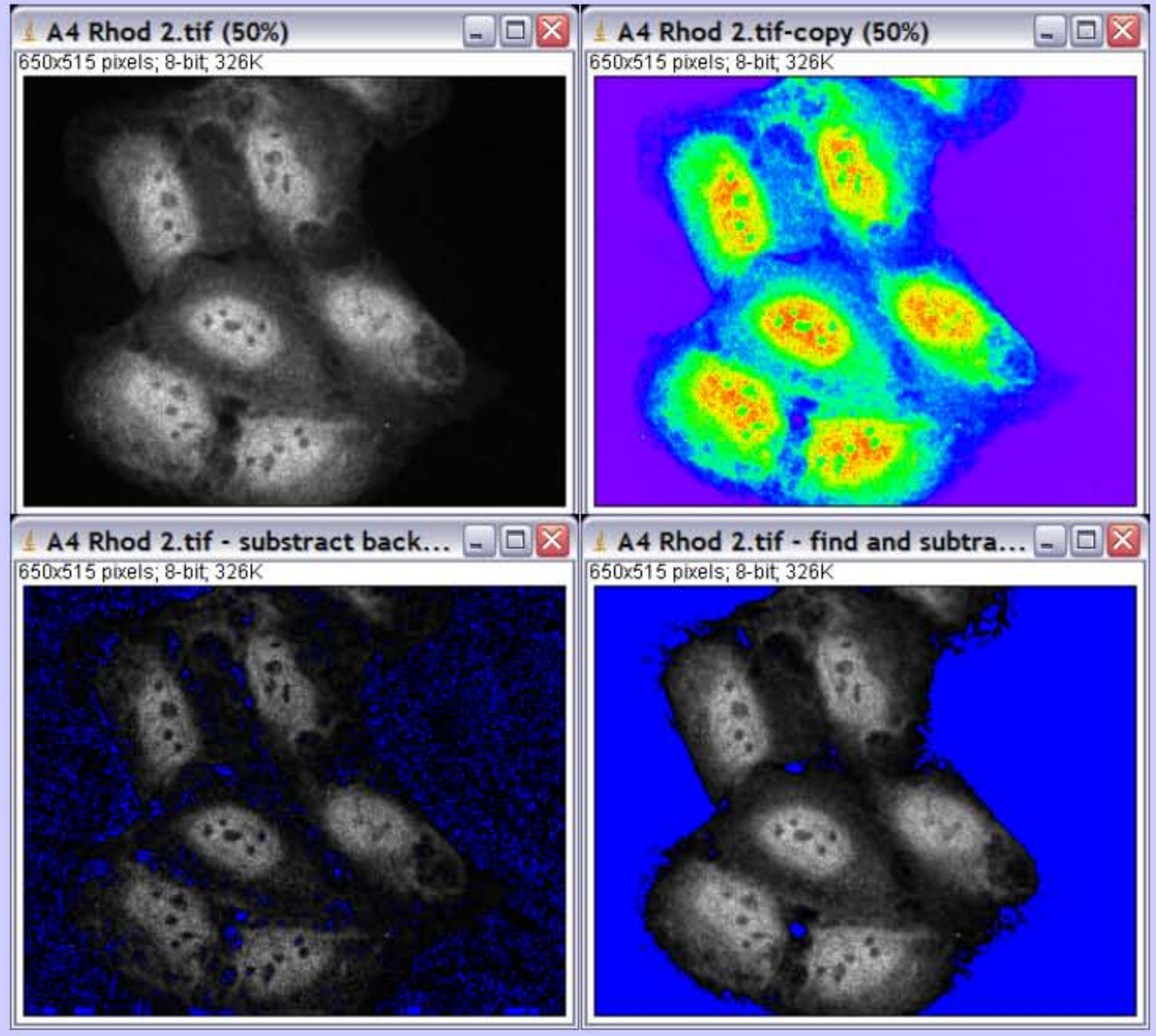


	A	B	C	D	E	F
1	**Image**	**Areas**	**Objects**	**Objects inside areas**	**ratio**	**folder**
2	hst-0.tif	3	891	52	0,06	C:\images\example 2\
3	hst-1.tif	4	918	89	0,1	C:\images\example 2\
4	hst-2.tif	3	942	61	0,06	C:\images\example 2\
5	hst-3.tif	4	899	70	0,08	C:\images\example 2\
6	hst-4.tif	9	966	116	0,12	C:\images\example 2\
7	hst.tif	3	315	17	0,05	C:\images\example 2\

Further interactive tools - LUT tool

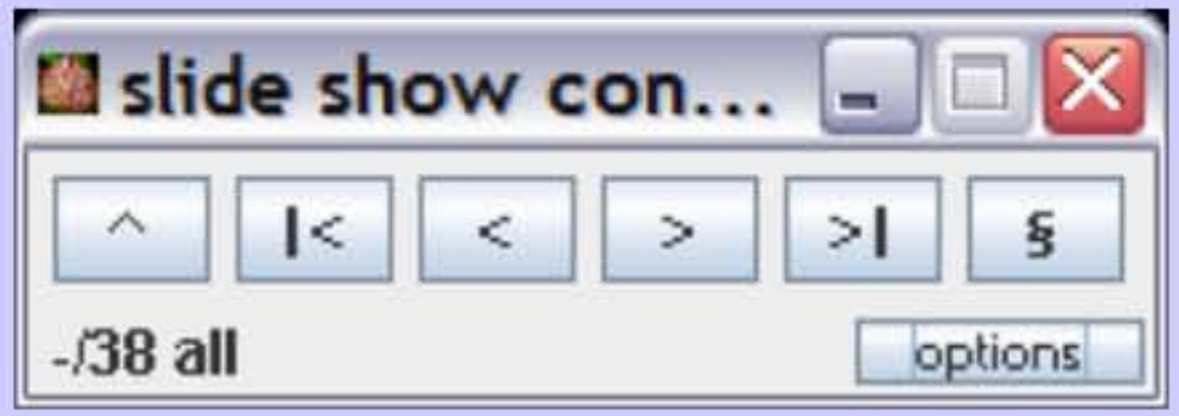


- apply lookup table to
  - active image
  - all open images

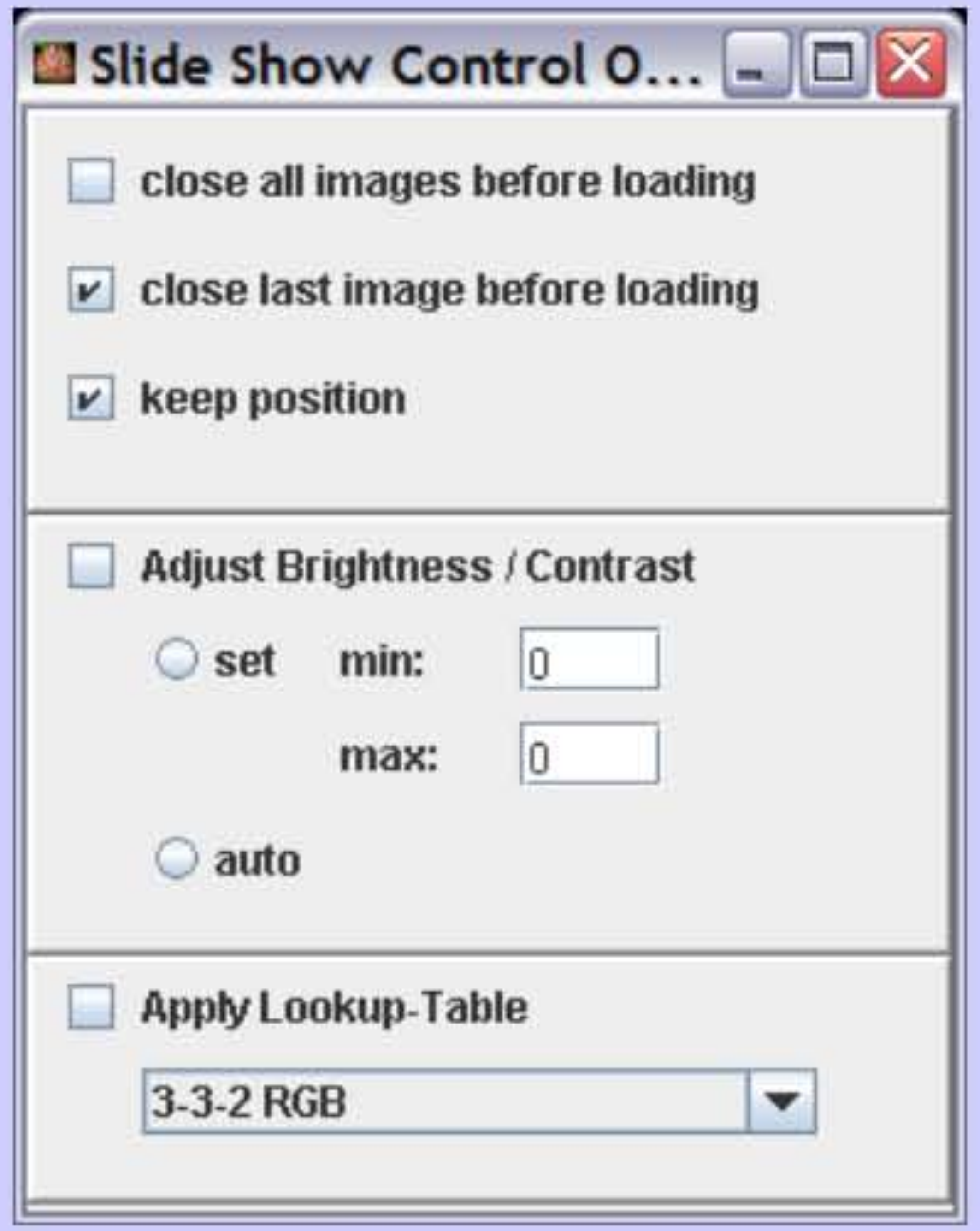

A4 Rhod 2.tif (50%)
650x515 pixels; 8-bit; 326K
A4 Rhod 2.tif-copy (50%)
650x515 pixels; 8-bit; 326K
A4 Rhod 2.tif - substract back...
650x515 pixels; 8-bit; 326K
A4 Rhod 2.tif - find and subtra...
650x515 pixels; 8-bit; 326K

1. gray
2. rainbow smooth
3. hilo after “subtract background”
4. hilo after “find and subtract” background

Further interactive tools – slide show control



-/38 all



□ close all images before loading

☑ close last image before loading

☑ keep position

□ Adjust Brightness / Contrast

○ set min: 0

max: 0

○ auto

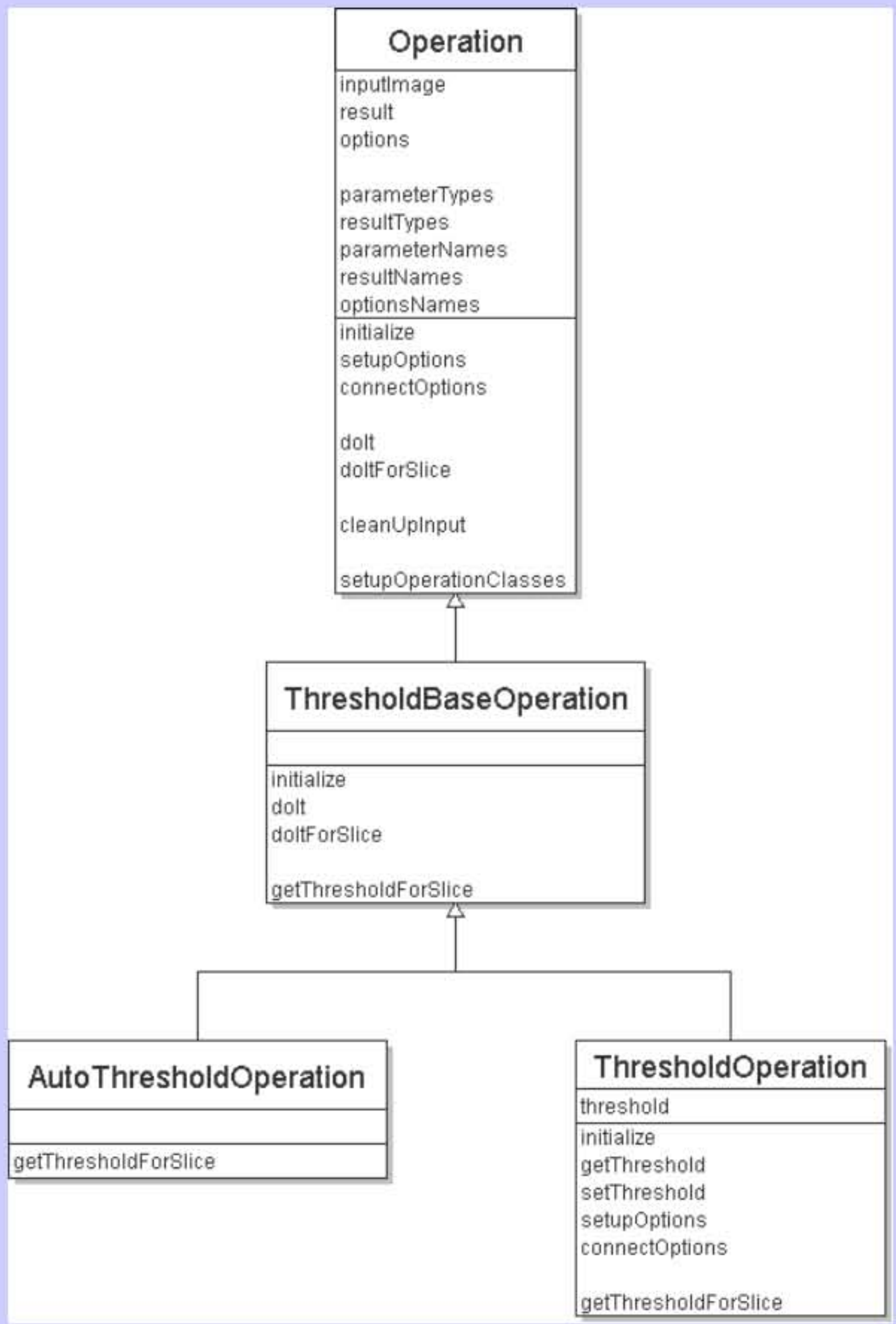
□ Apply Lookup-Table

3-3-2 RGB

- **navigation**
  - select folder
  - goto first image
  - goto last image
  - goto next image
  - goto last image
  - reload current image
- **when opening next image**
  - close all open images
  - close last image opened with slide show control
  - keep position and zoom
  - adjust brightness/contrast (fix or auto)
  - apply LUT

Extending the framework

- to add an operation (without options)
  - declare input parameter and result in initialize
  - implement
    - doIt
    - doItForSlice



Operation

inputImage
result
options

parameterTypes
resultTypes
parameterNames
resultNames
optionsNames

initialize
setupOptions
connectOptions

doIt
doItForSlice

cleanUpInput

setupOperationClasses

ThresholdBaseOperation

initialize
doIt
doItForSlice

getThresholdForSlice

AutoThresholdOperation

getThresholdForSlice

ThresholdOperation

threshold

initialize
getThreshold
setThreshold
setupOptions
connectOptions

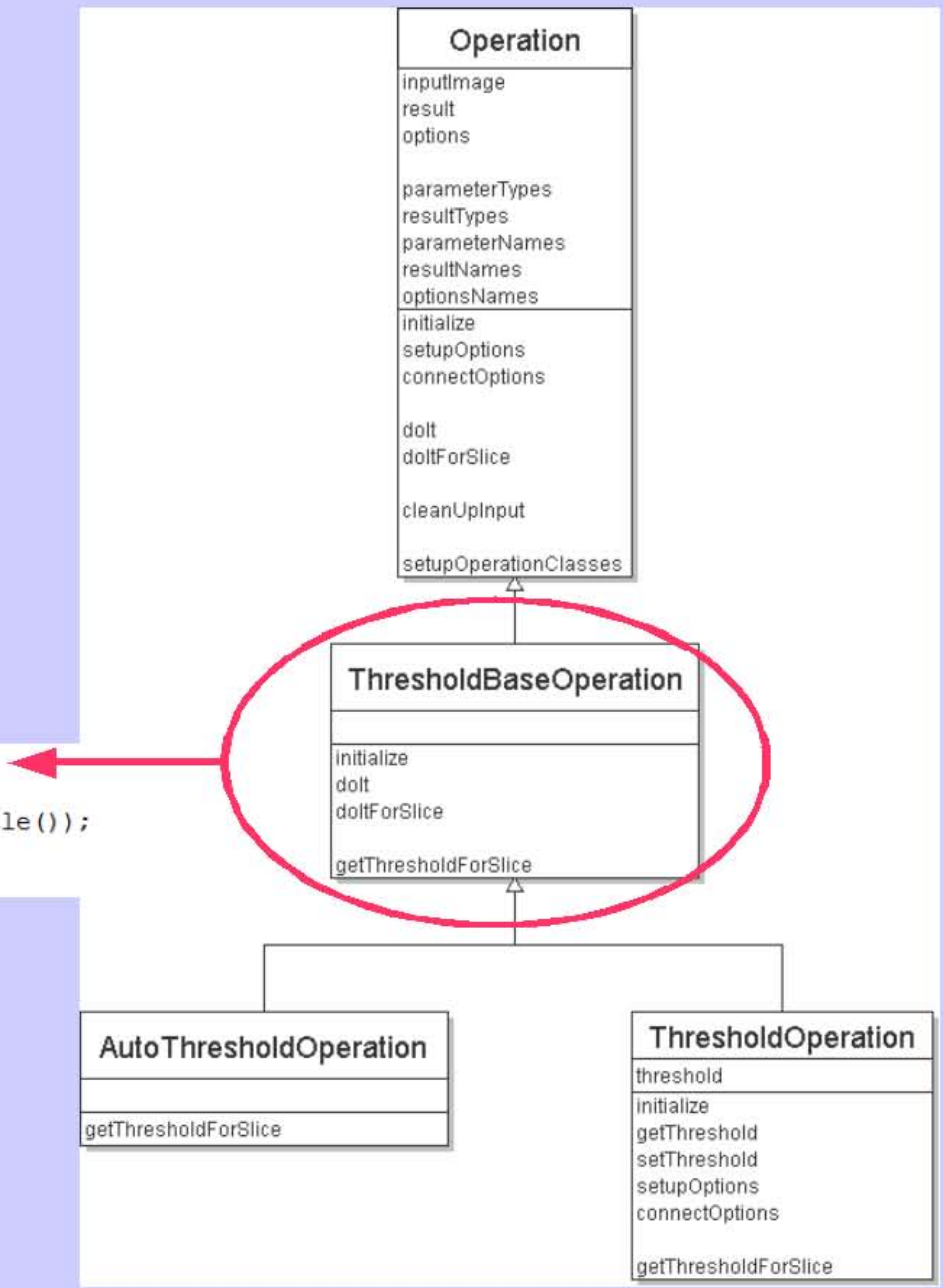
getThresholdForSlice

Extending the framework

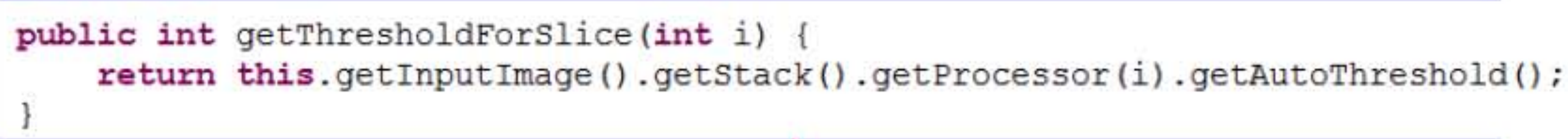
```java
protected void initialize() throws ClassNotFoundException {
    super.initialize();
    parameterTypes = new Class[1];
    parameterTypes[0] = Class.forName("ij.ImagePlus");
    parameterNames = new String[1];
    parameterNames[0] = "InputImage";
    resultTypes = new Class[1];
    resultTypes[0] = Class.forName("ij.ImagePlus");
    resultNames = new String[1];
    resultNames[0] = "Result";
}
```

```java
public void doIt() {
    ImagePlus inputImage = this.getInputImage();
    result = this.getCopyOfOrReferenceTo(inputImage, inputImage.getTitle());
    this.processSlices();
}
```

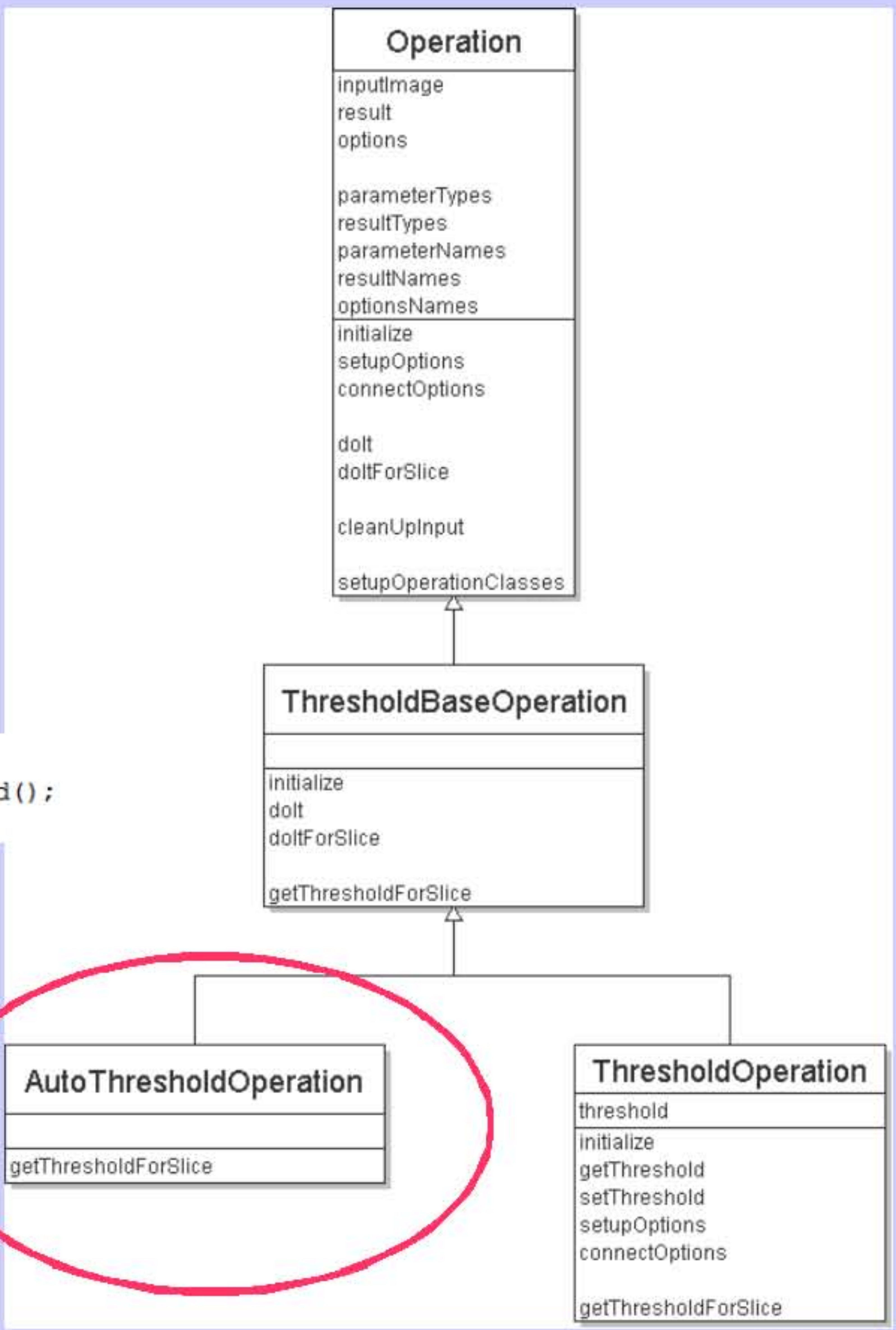
```java
protected void doItForSlice(int sliceNumber, ImageStack stack) {
    ImageProcessor ip = stack.getProcessor(sliceNumber);
    ip.threshold(this.getThresholdForSlice(sliceNumber));
}
```



Extending the framework



```
public int getThresholdForSlice(int i) {
    return this.getInputImage().getStack().getProcessor(i).getAutoThreshold();
}
```



Operation
inputImage
result
options

parameterTypes
resultTypes
parameterNames
resultNames
optionsNames

initialize
setupOptions
connectOptions

doIt
doItForSlice

cleanUpInput

setupOperationClasses

ThresholdBaseOperation

initialize
doIt
doItForSlice

getThresholdForSlice

AutoThresholdOperation

getThresholdForSlice

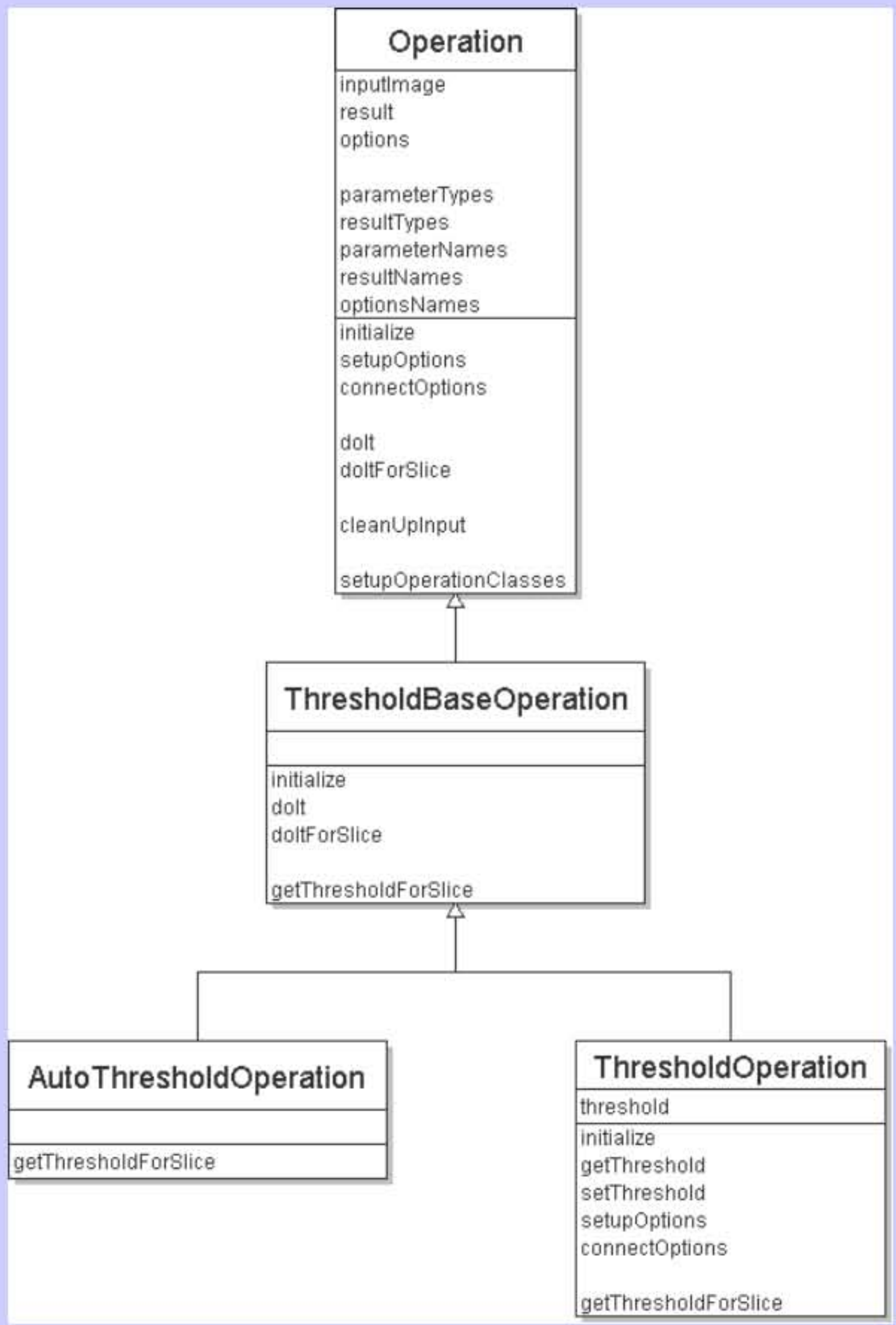
ThresholdOperation
threshold

initialize
getThreshold
setThreshold
setupOptions
connectOptions

getThresholdForSlice

Extending the framework

- to add an operation with options
  - declare options in initialize
  - setup options
    - set name, default value, min, max and short help text
  - to facilitate access
    - declare instance variable
    - connectOptions
    - getter / setter



Operation

inputImage
result
options

parameterTypes
resultTypes
parameterNames
resultNames
optionsNames

initialize
setupOptions
connectOptions

doIt
doItForSlice

cleanUpInput

setupOperationClasses

ThresholdBaseOperation

initialize
doIt
doItForSlice

getThresholdForSlice

AutoThresholdOperation

getThresholdForSlice

ThresholdOperation

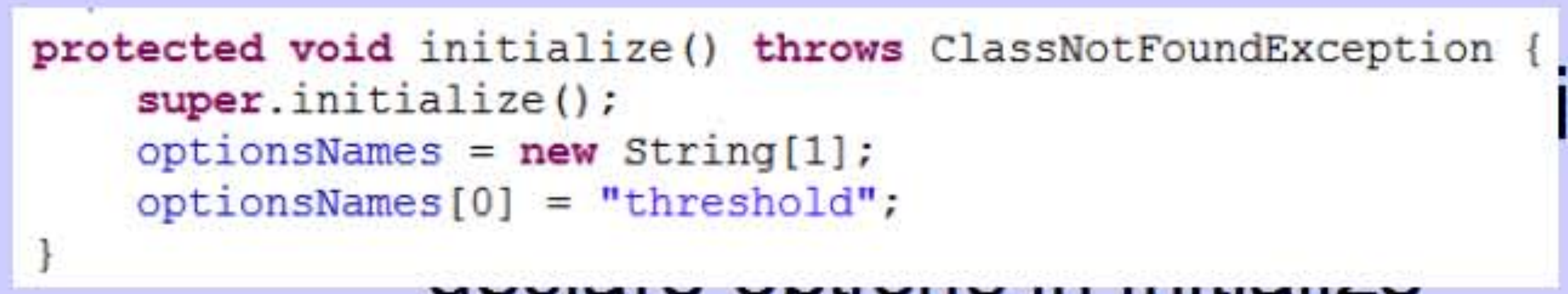
threshold

initialize
getThreshold
setThreshold
setupOptions
connectOptions

getThresholdForSlice

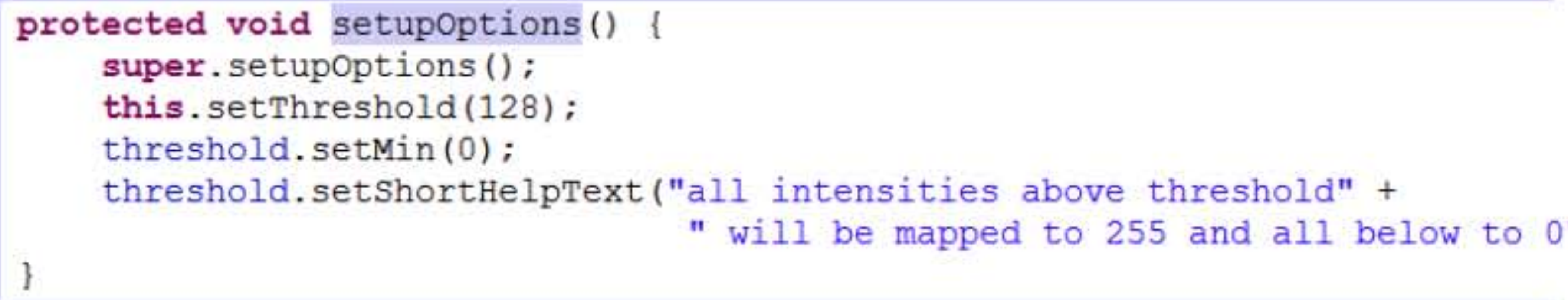
Extending the framework

ions



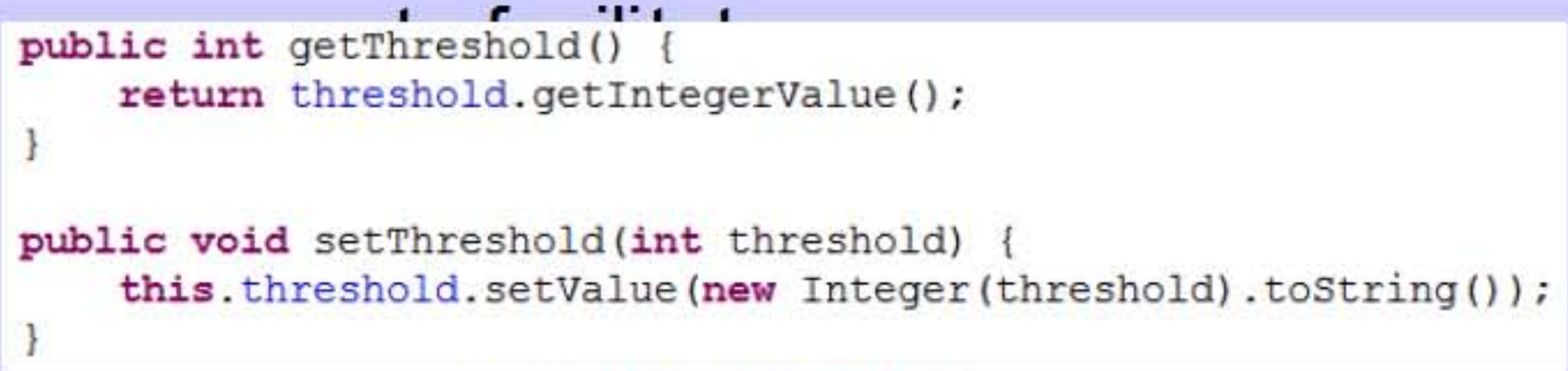
```
protected void initialize() throws ClassNotFoundException {
    super.initialize();
    optionsNames = new String[1];
    optionsNames[0] = "threshold";
}
```

declare options in initialize



```
protected void setupOptions() {
    super.setupOptions();
    this.setThreshold(128);
    threshold.setMin(0);
    threshold.setShortHelpText("all intensities above threshold" +
                             " will be mapped to 255 and all below to 0");
}
```

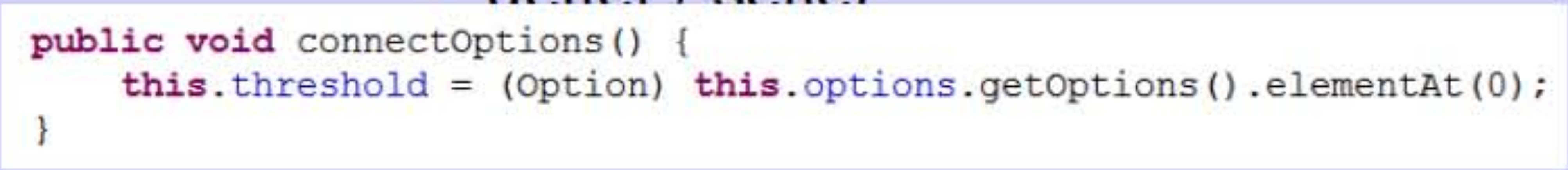
to facilitat



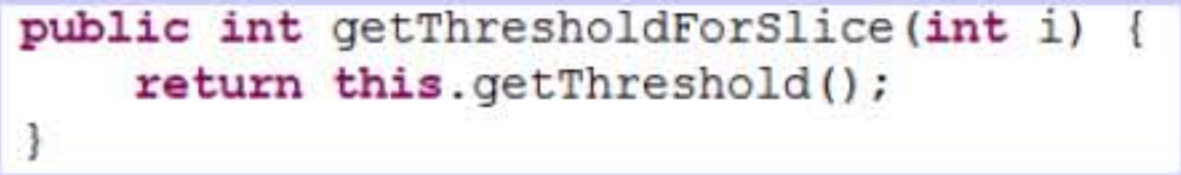
```
public int getThreshold() {
    return threshold.getIntegerValue();
}

public void setThreshold(int threshold) {
    this.threshold.setValue(new Integer(threshold).toString());
}
```

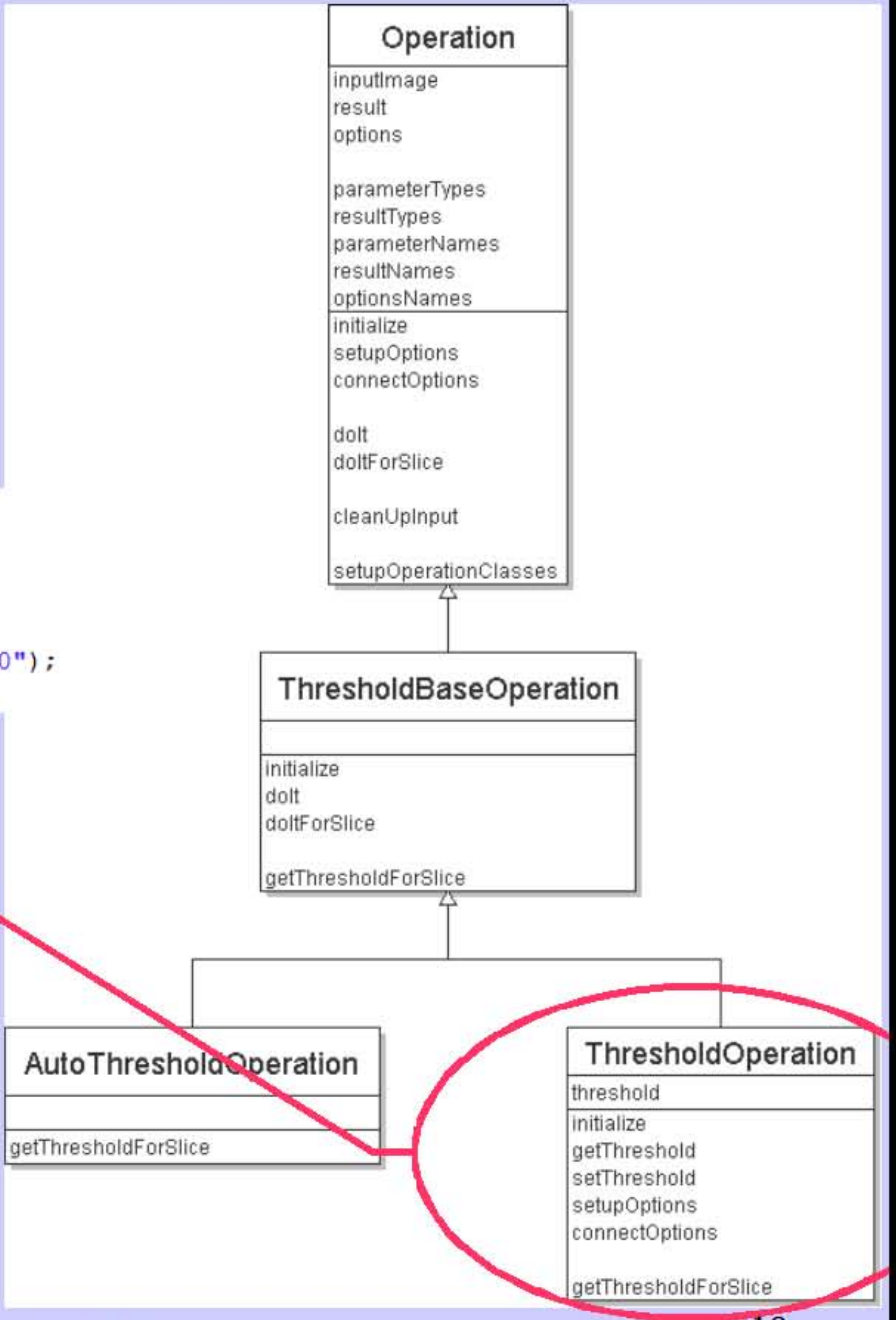
- getter / setter



```
public void connectOptions() {
    this.threshold = (Option) this.options.getOptions().elementAt(0);
}
```

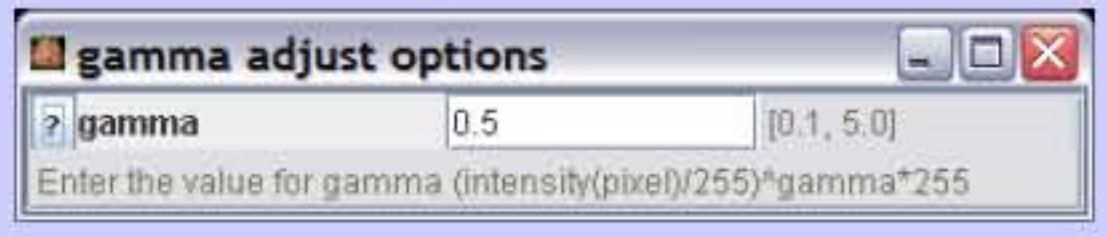


```
public int getThresholdForSlice(int i) {
    return this.getThreshold();
}
```



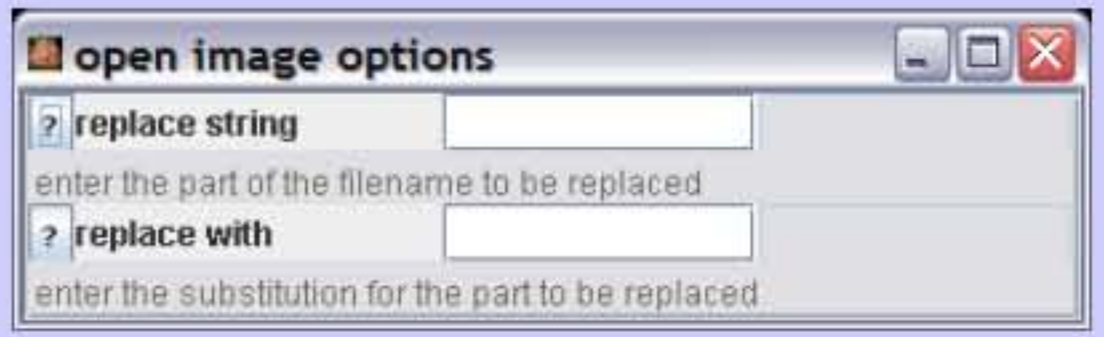
Options

Option


gamma adjust options

? gamma 0.5 [0.1, 5.0]

Enter the value for gamma (intensity(pixel)/255)^gamma*255

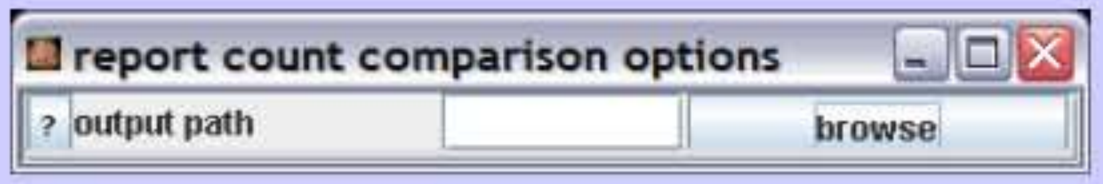

open image options

? replace string

enter the part of the filename to be replaced

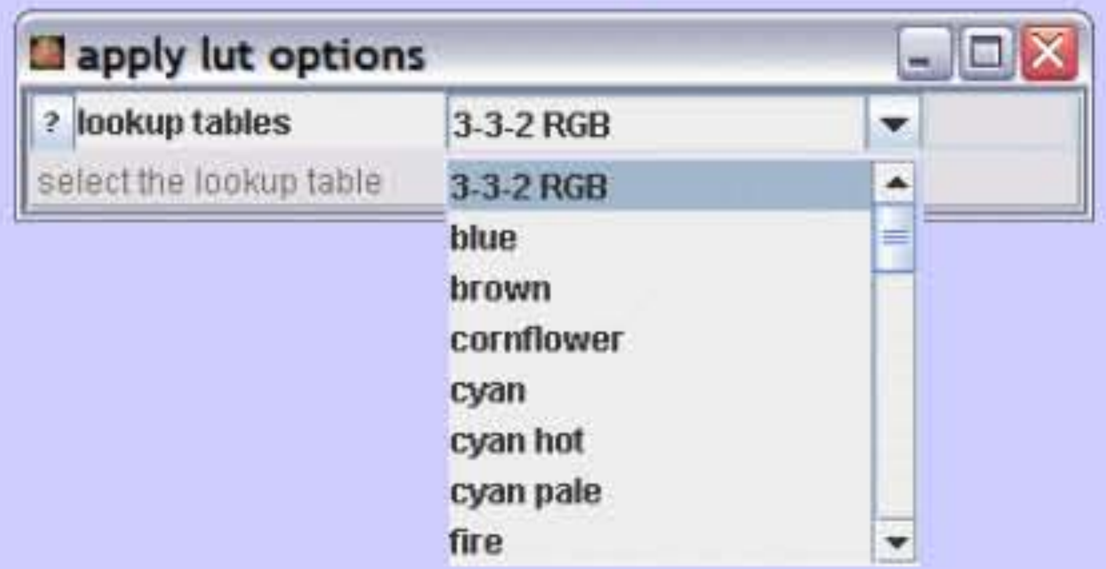
? replace with

enter the substitution for the part to be replaced


report count comparison options

? output path

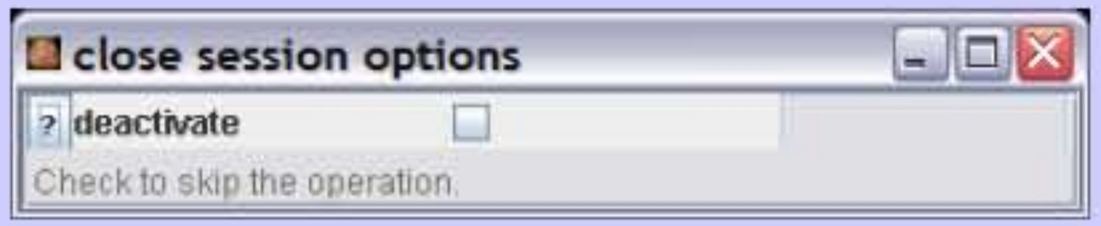
ChoiceOption


apply lut options

? lookup tables 3-3-2 RGB

select the lookup table

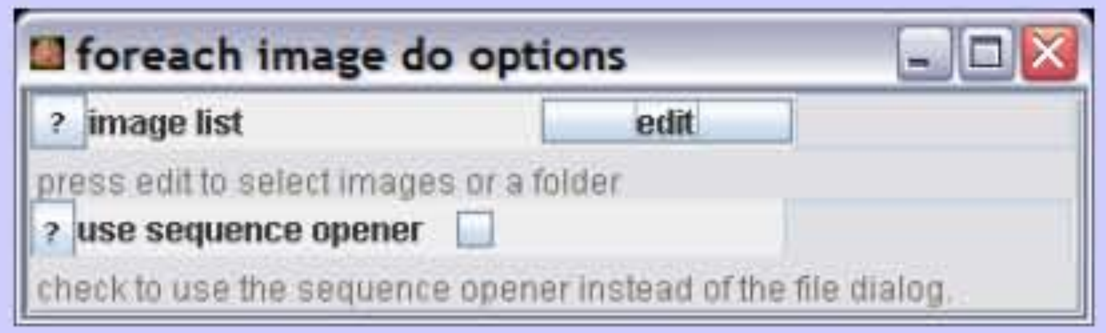
BooleanOption


close session options

? deactivate ☐

Check to skip the operation.

ListOption


foreach image do options

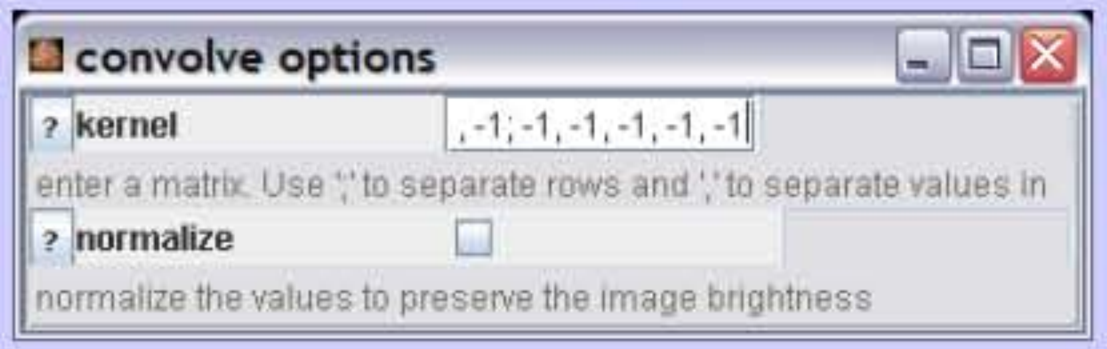
? image list

press edit to select images or a folder

? use sequence opener ☐

check to use the sequence opener instead of the file dialog.

MatrixOption

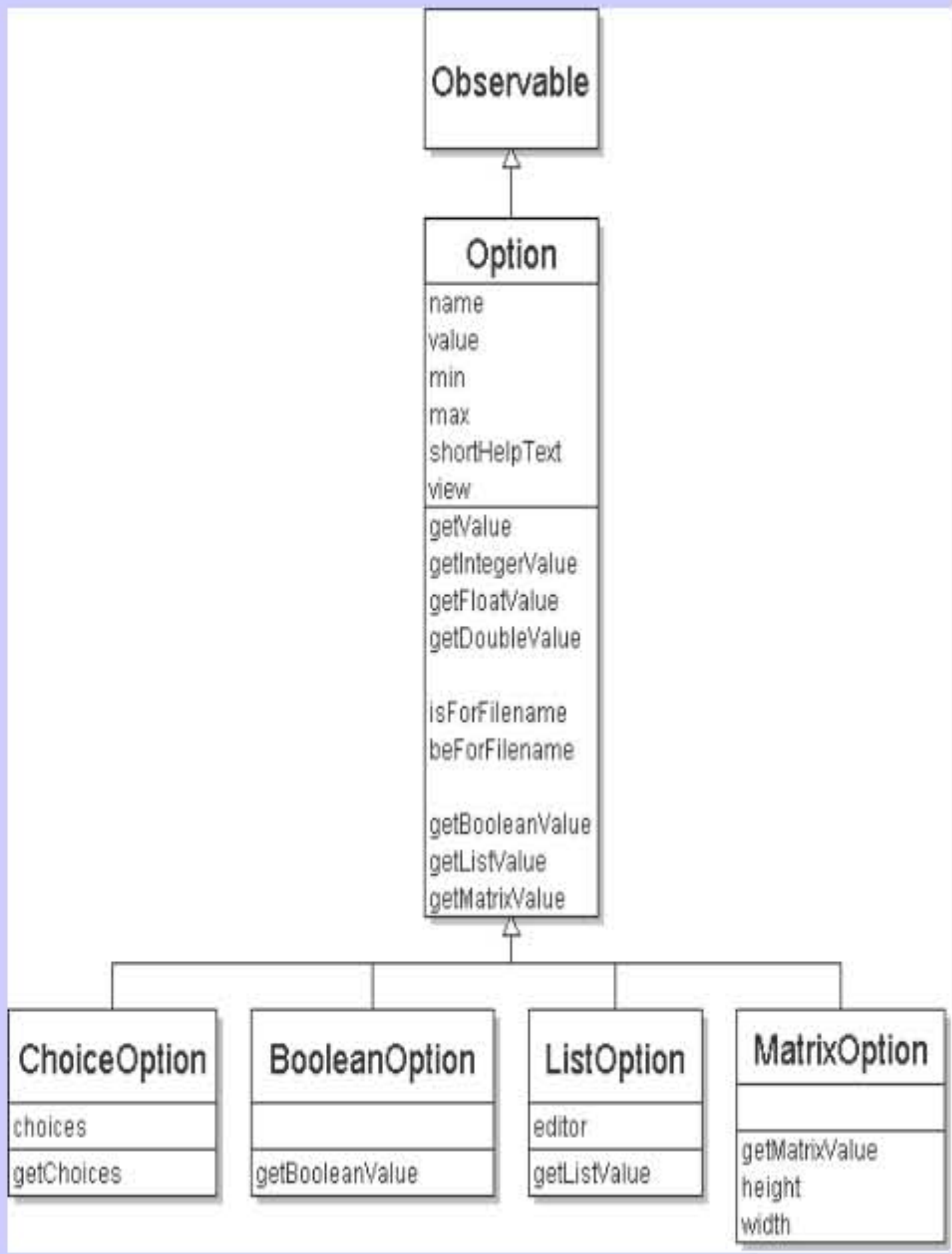

convolve options

? kernel ,-1;-1,-1,-1,-1,-1

enter a matrix. Use ';' to separate rows and ',' to separate values in

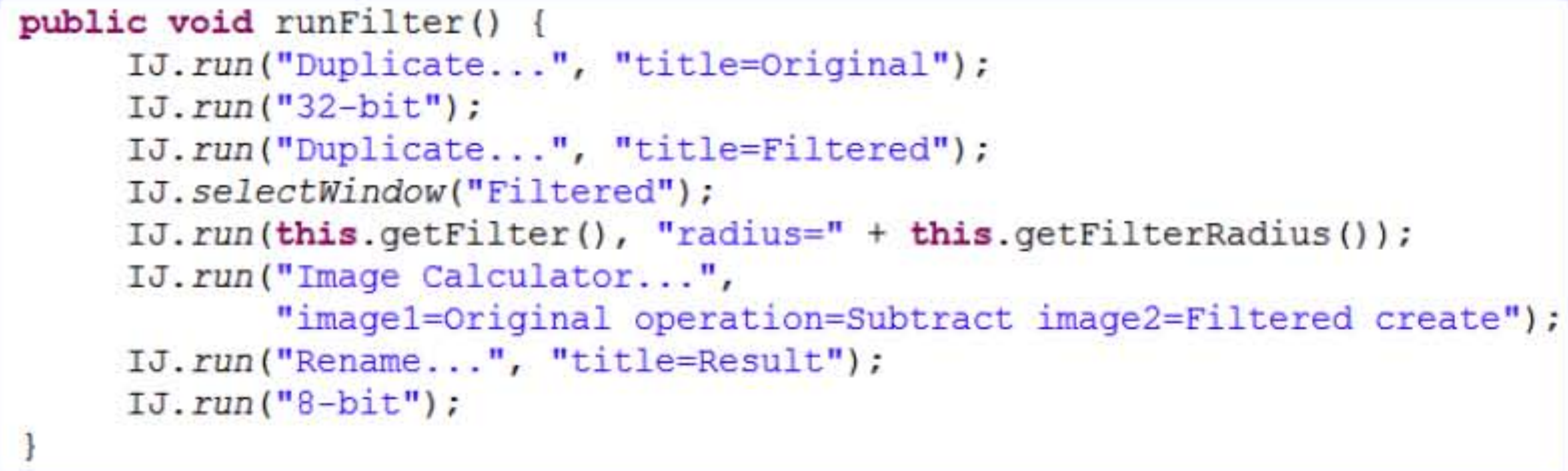
? normalize ☐

normalize the values to preserve the image brightness

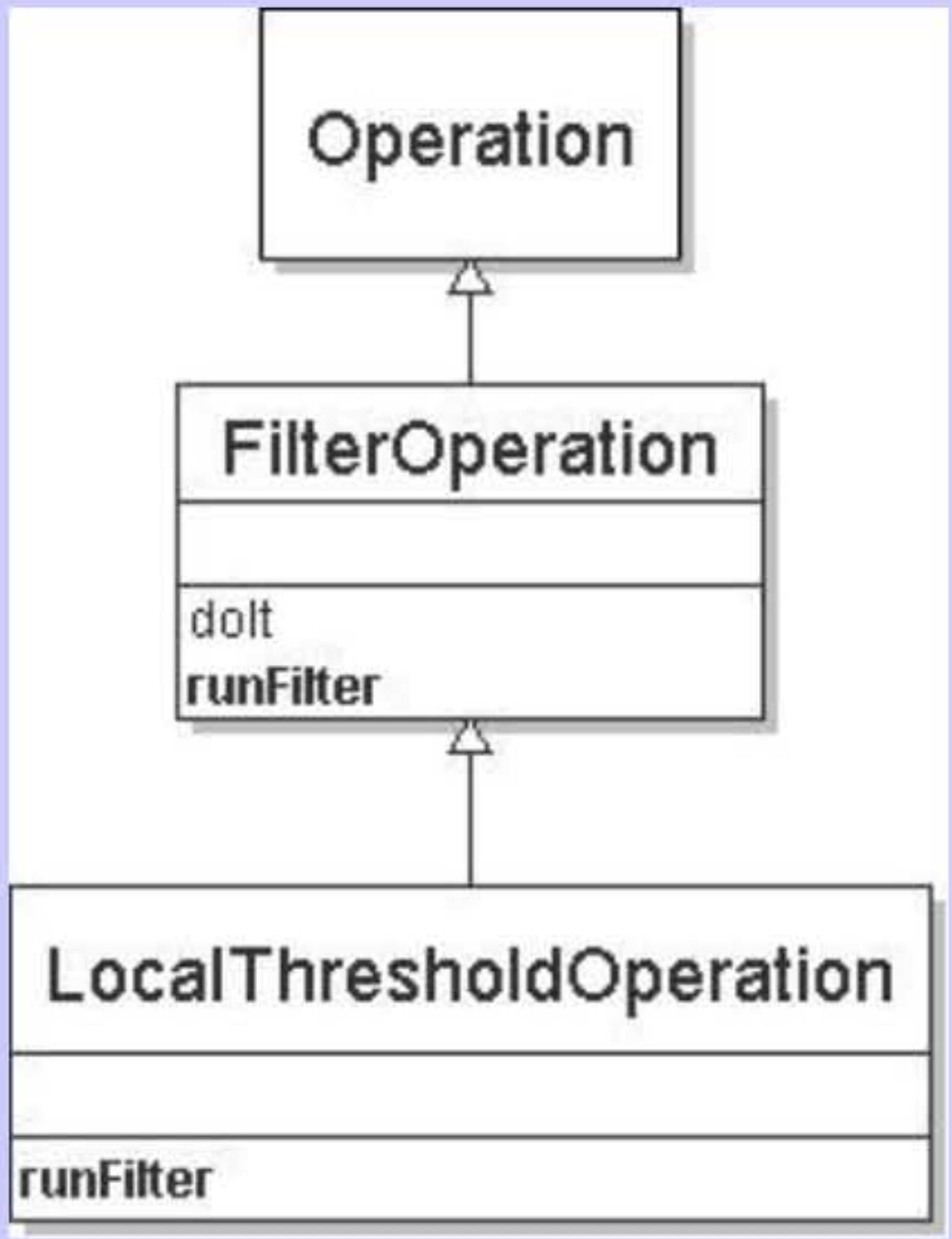


Accessing ImageJ methods

- simplest method:
- use the ImageJ macros interface
  - IJ.run(...)



```
public void runFilter() {
     IJ.run("Duplicate...", "title=Original");
     IJ.run("32-bit");
     IJ.run("Duplicate...", "title=Filtered");
     IJ.selectWindow("Filtered");
     IJ.run(this.getFilter(), "radius=" + this.getFilterRadius());
     IJ.run("Image Calculator...",
            "image1=Original operation=Subtract image2=Filtered create");
     IJ.run("Rename...", "title=Result");
     IJ.run("8-bit");
}
```



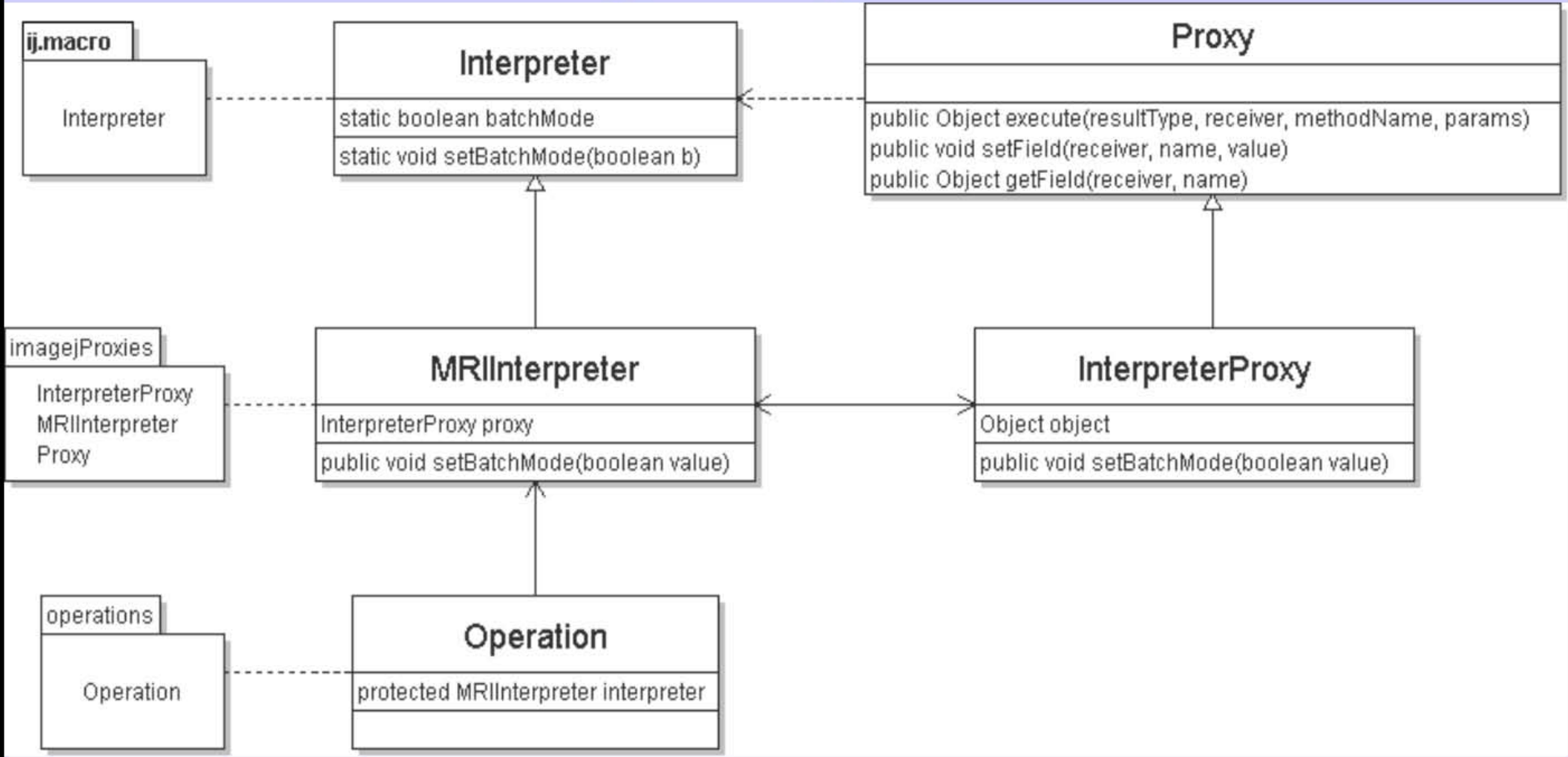
Local threshold macro by R.Couture 11-14-03 Dept. of Radiology Washington University School of Medicine, couture@wuerl.wustl.edu

Accessing ImageJ methods

- what if method or data member not accessible
  - access to protected:
    - subclass in own package
  - access to private:
    - use java reflection api

- class Proxy facilitates reflection api usage
  - Object execute(...)
  - void setField(...)
  - Object getField(...)

Accessing ImageJ methods



ij.macro

Interpreter

Interpreter

static boolean batchMode

static void setBatchMode(boolean b)

Proxy

public Object execute(resultType, receiver, methodName, params)
public void setField(receiver, name, value)
public Object getField(receiver, name)

imagejProxies

InterpreterProxy
MRIInterpreter
Proxy

MRIInterpreter

InterpreterProxy proxy

public void setBatchMode(boolean value)

InterpreterProxy

Object object

public void setBatchMode(boolean value)

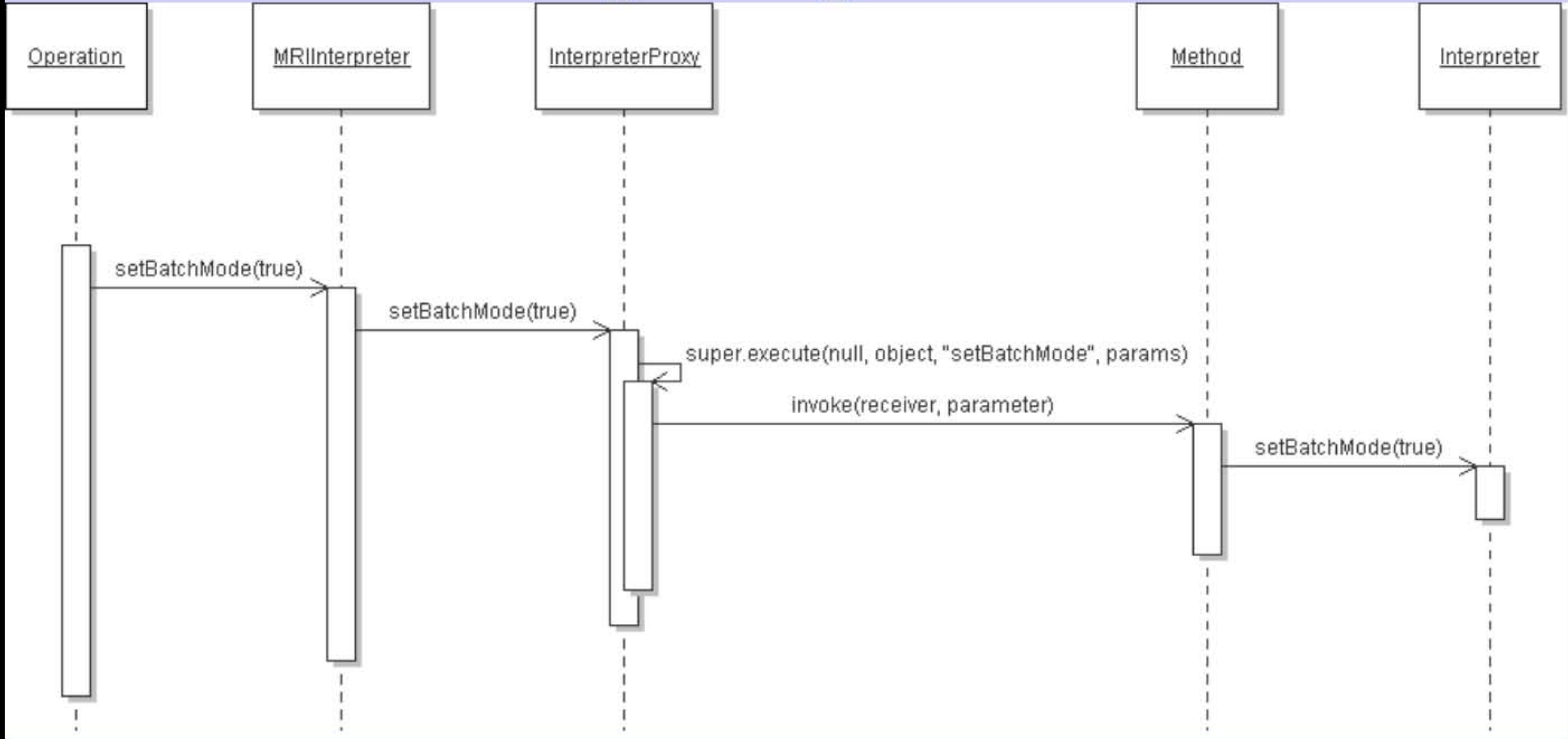
operations

Operation

Operation

protected MRIInterpreter interpreter

Accessing ImageJ methods



Operation

MRIInterpreter

InterpreterProxy

Method

Interpreter

setBatchMode(true)

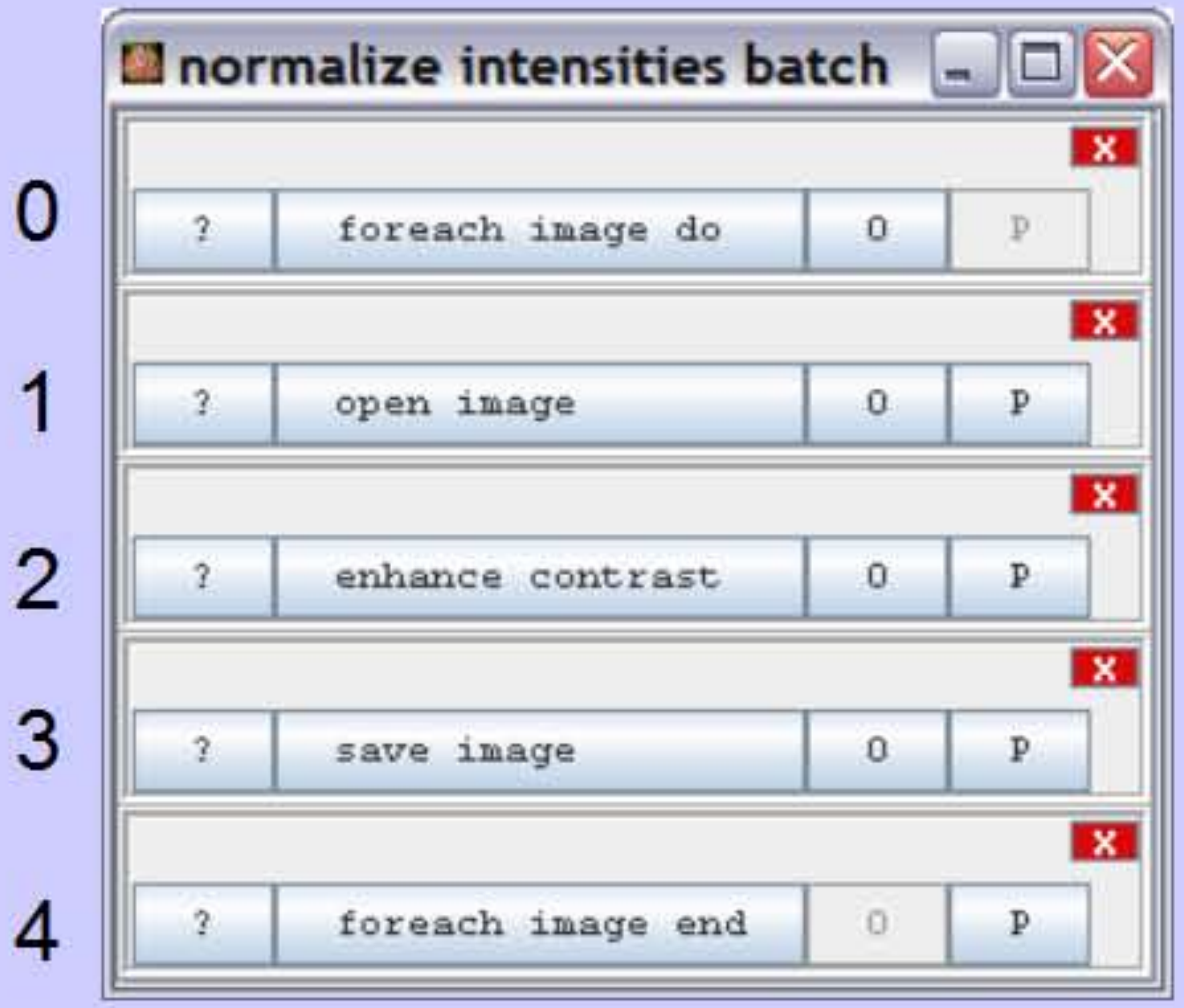
setBatchMode(true)

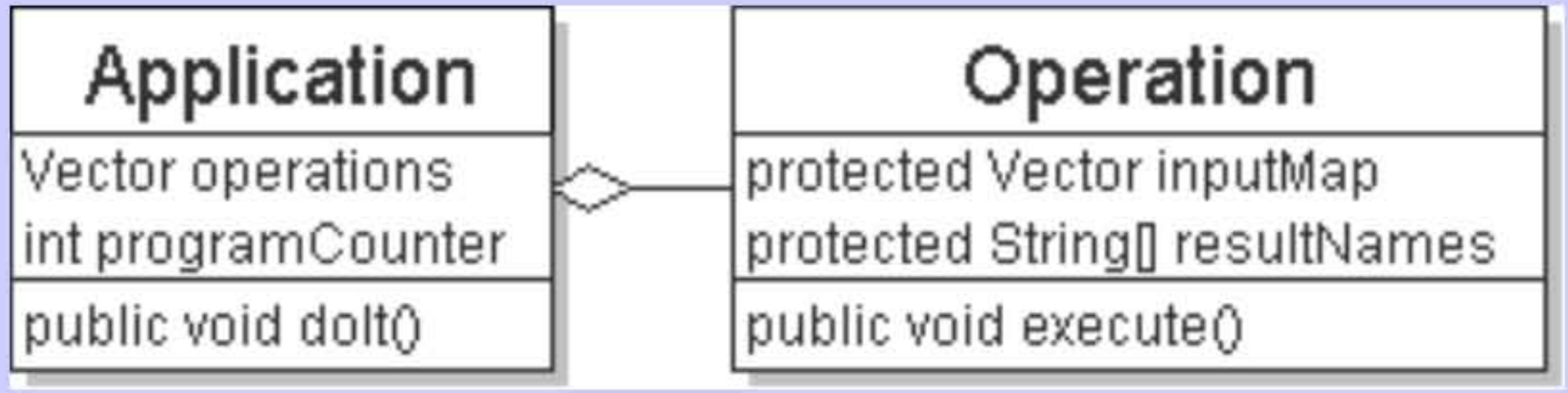
super.execute(null, object, "setBatchMode", params)

invoke(receiver, parameter)

setBatchMode(true)

The implementation





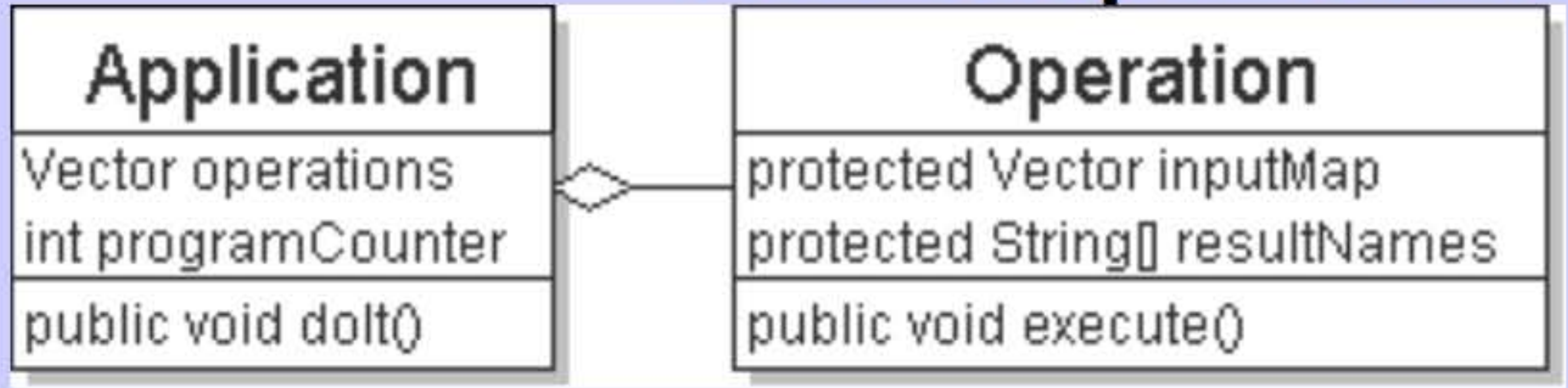
Application
Vector operations int programCounter
public void doIt()

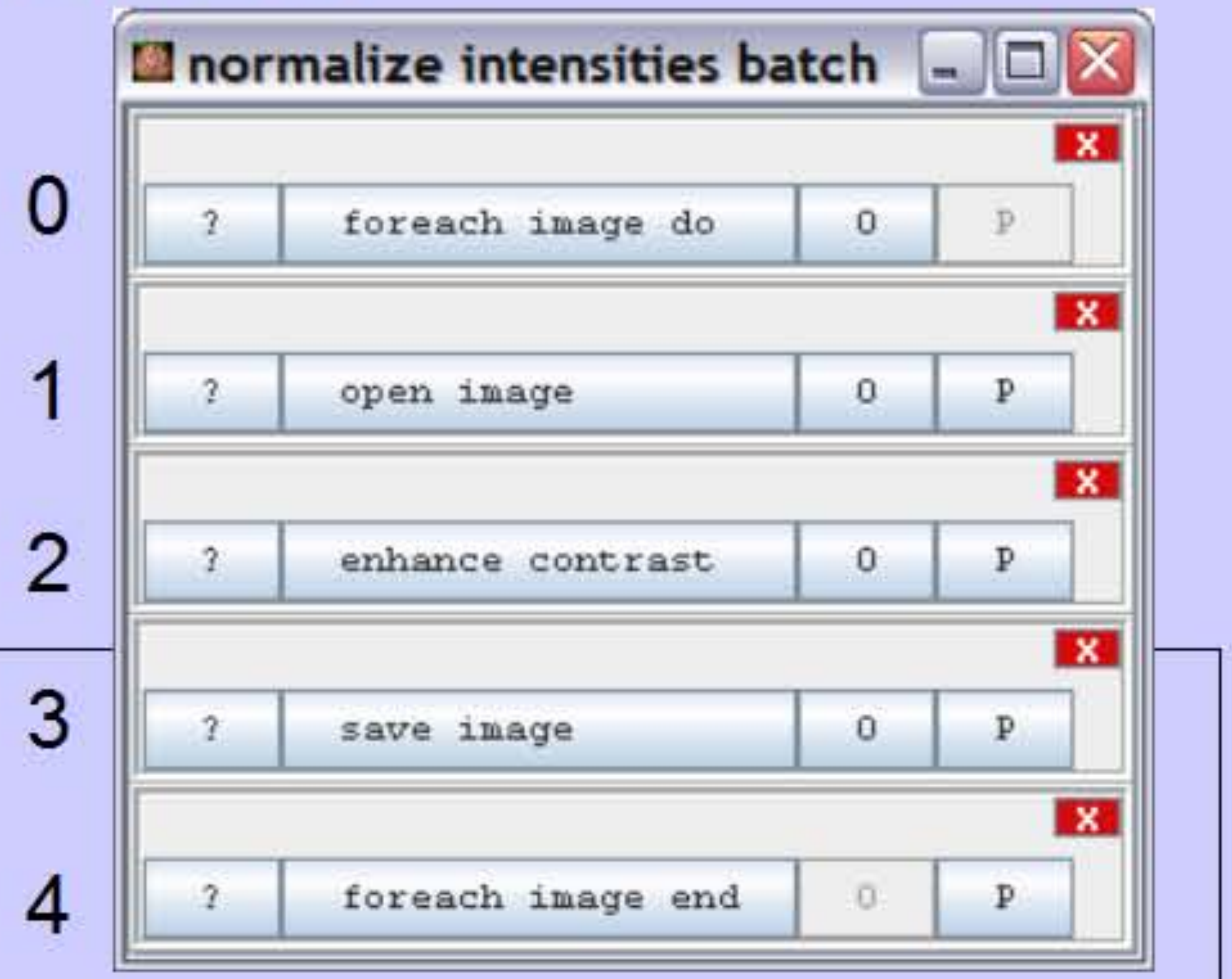
Operation
protected Vector inputMap protected String[] resultNames
public void execute()

```
input maps:
   operation    result nr.
0:     -            -
1:     0            0
2:     1            0
3:     2            0
       0            0
4:     0            2
```

- When application is loaded from text file
  - objects are created from names, using reflection
  - parameters of operation are connected to results of preceding operations

The implementation



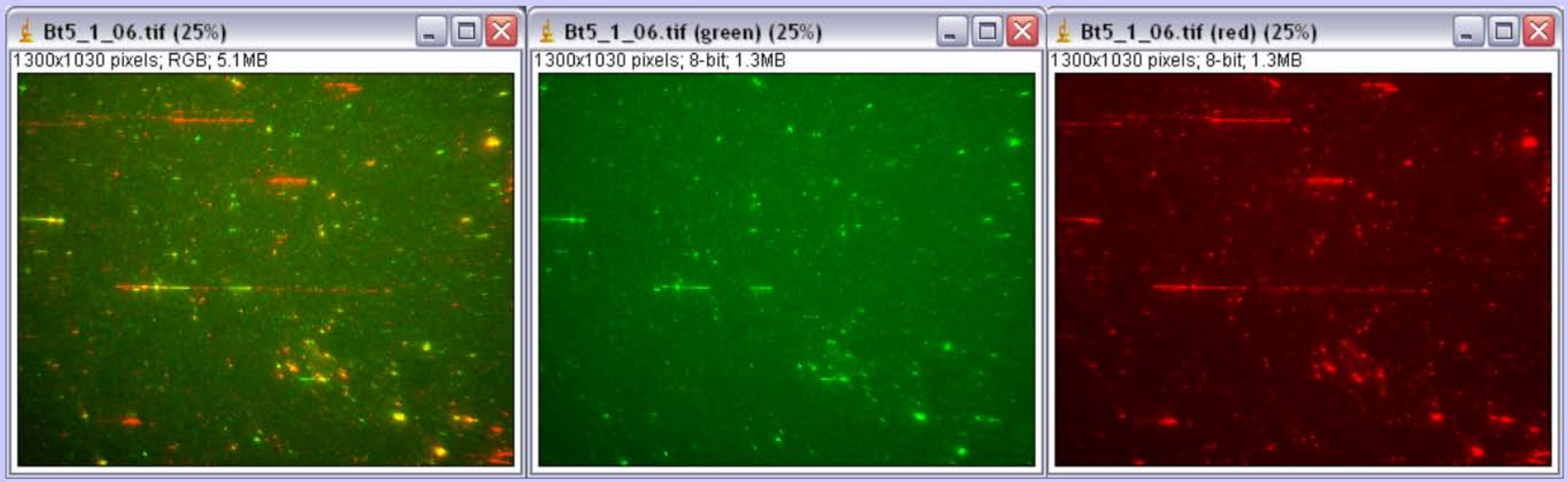


```
Application>>doIt

FOR programCounter = 0 TO numberOfOperations - 1
    if (stopped) return
    execute operation
    cleanup preceding results not referenced afterwards
    get next operation
    set parameters of next operation from results of preceding operations, using reflection
```

jump and loop operations modify the programCounter

DNA combing


Bt5_1_06.tif (25%)
1300x1030 pixels; RGB; 5.1MB
Bt5_1_06.tif (green) (25%)
1300x1030 pixels; 8-bit; 1.3MB
Bt5_1_06.tif (red) (25%)
1300x1030 pixels; 8-bit; 1.3MB

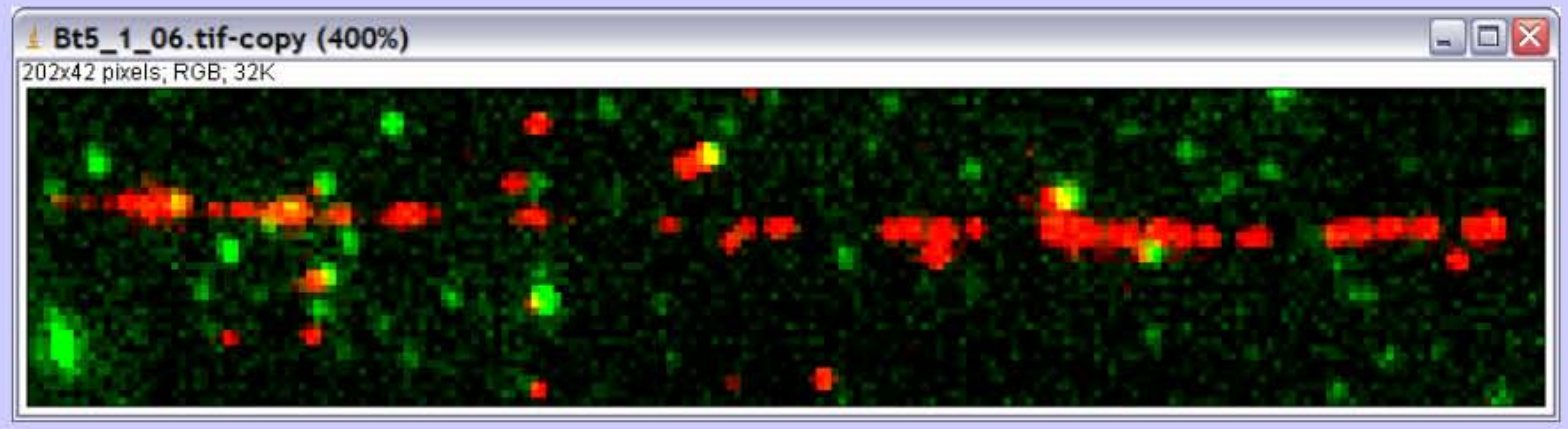
the images:

red:	combed DNA
green:	sites where replication takes place

the task, measure:

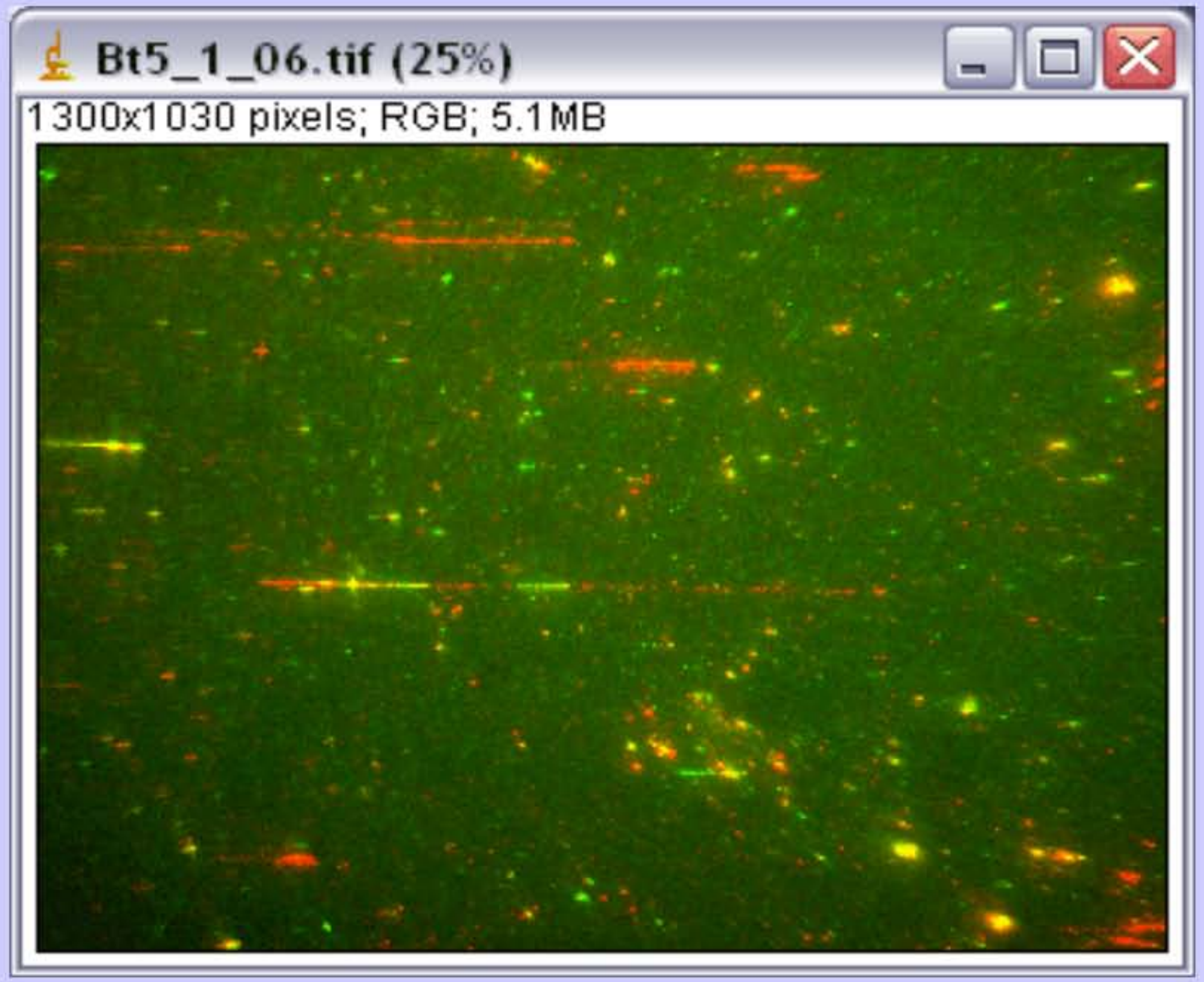
- the lengths of the DNA molecules
- the lengths of the replication sites within each DNA molecule
- the distances between replication sites for each DNA molecule

DNA combing


Bt5_1_06.tif-copy (400%)
202x42 pixels; RGB; 32K

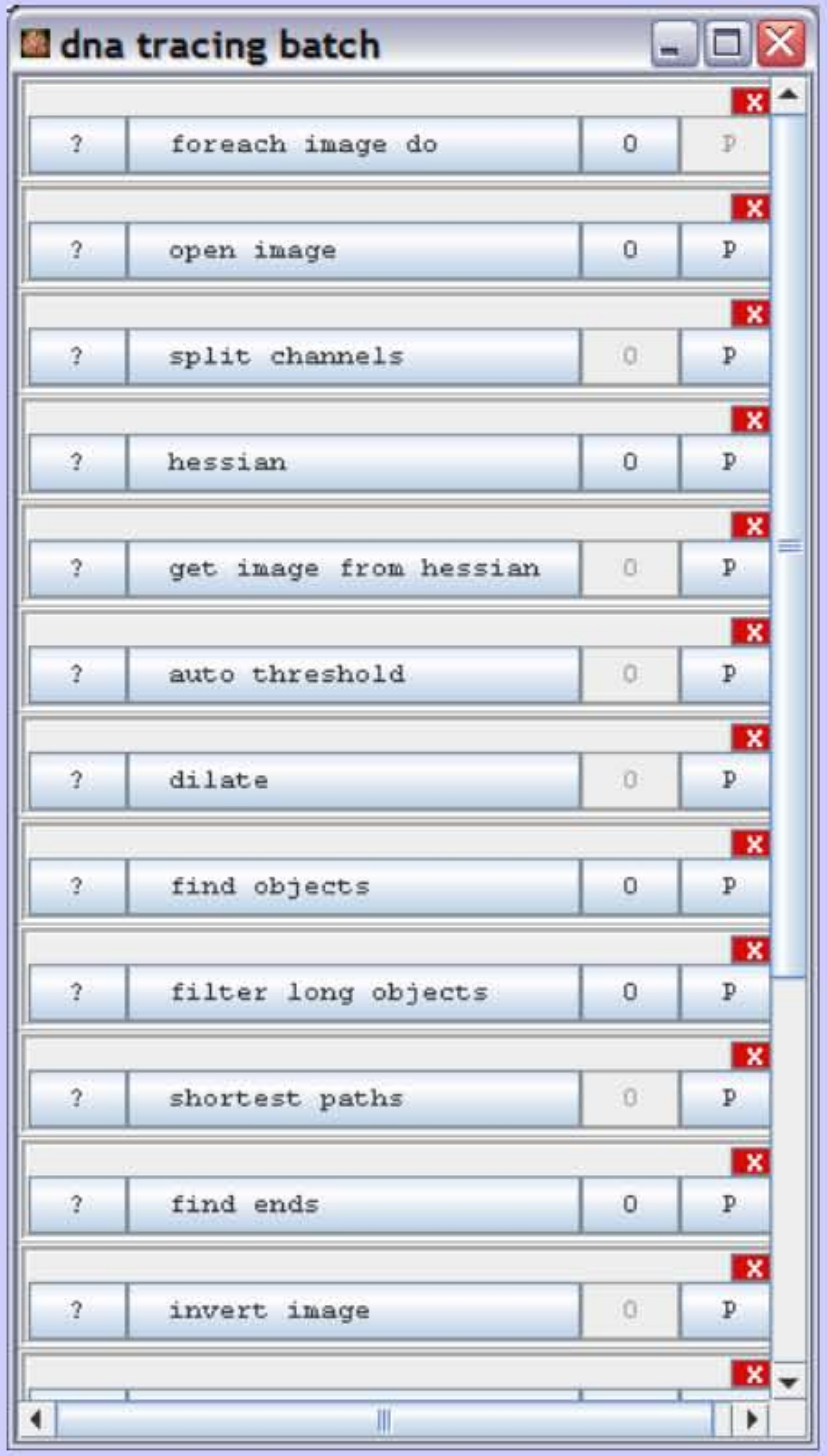
- difficulties
  - high level of (biochemical) noise
  - stretched out molecules are not straight
  - signal has large gaps

DNA combing



Bt5_1_06.tif (25%)

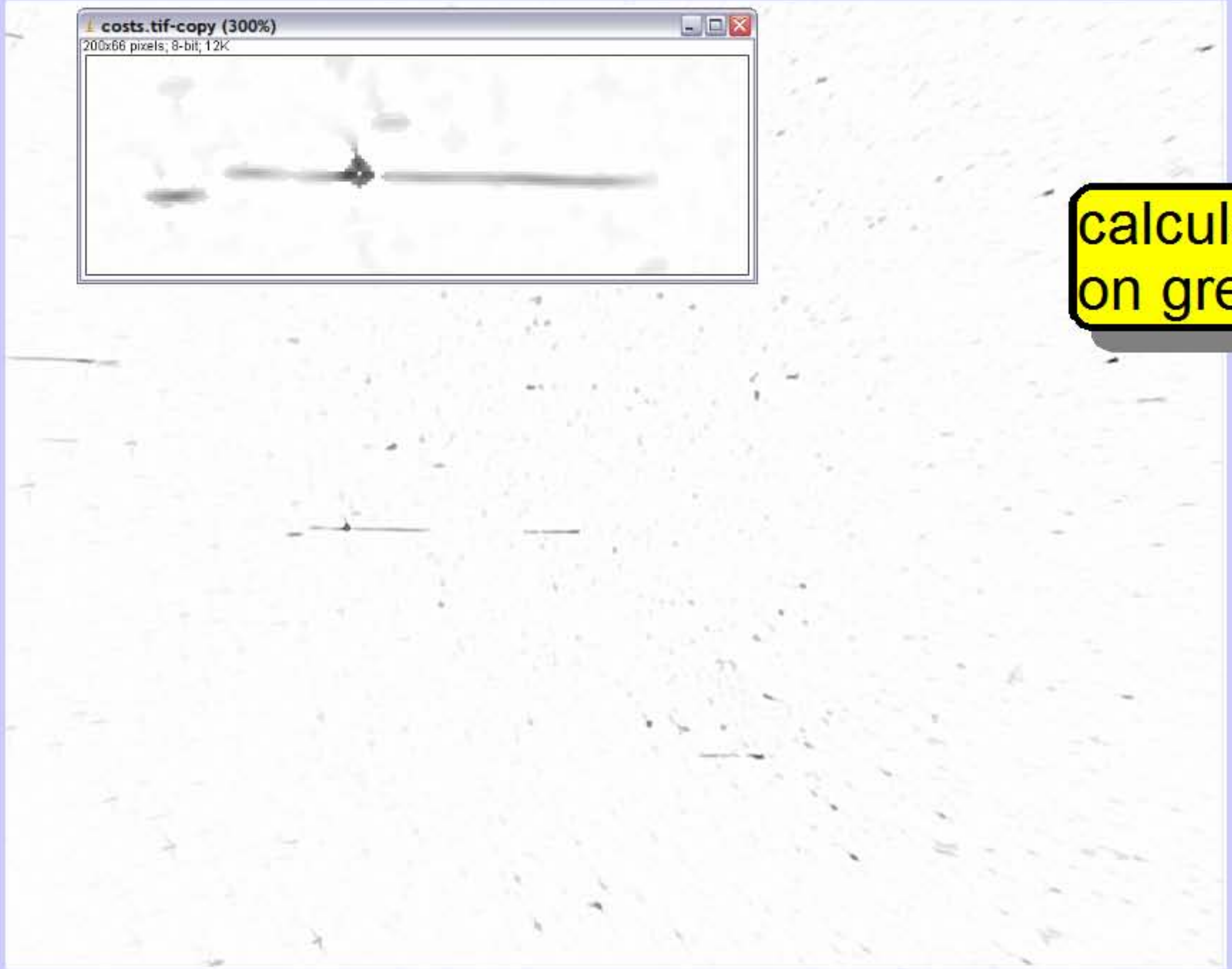
1300x1030 pixels; RGB; 5.1MB

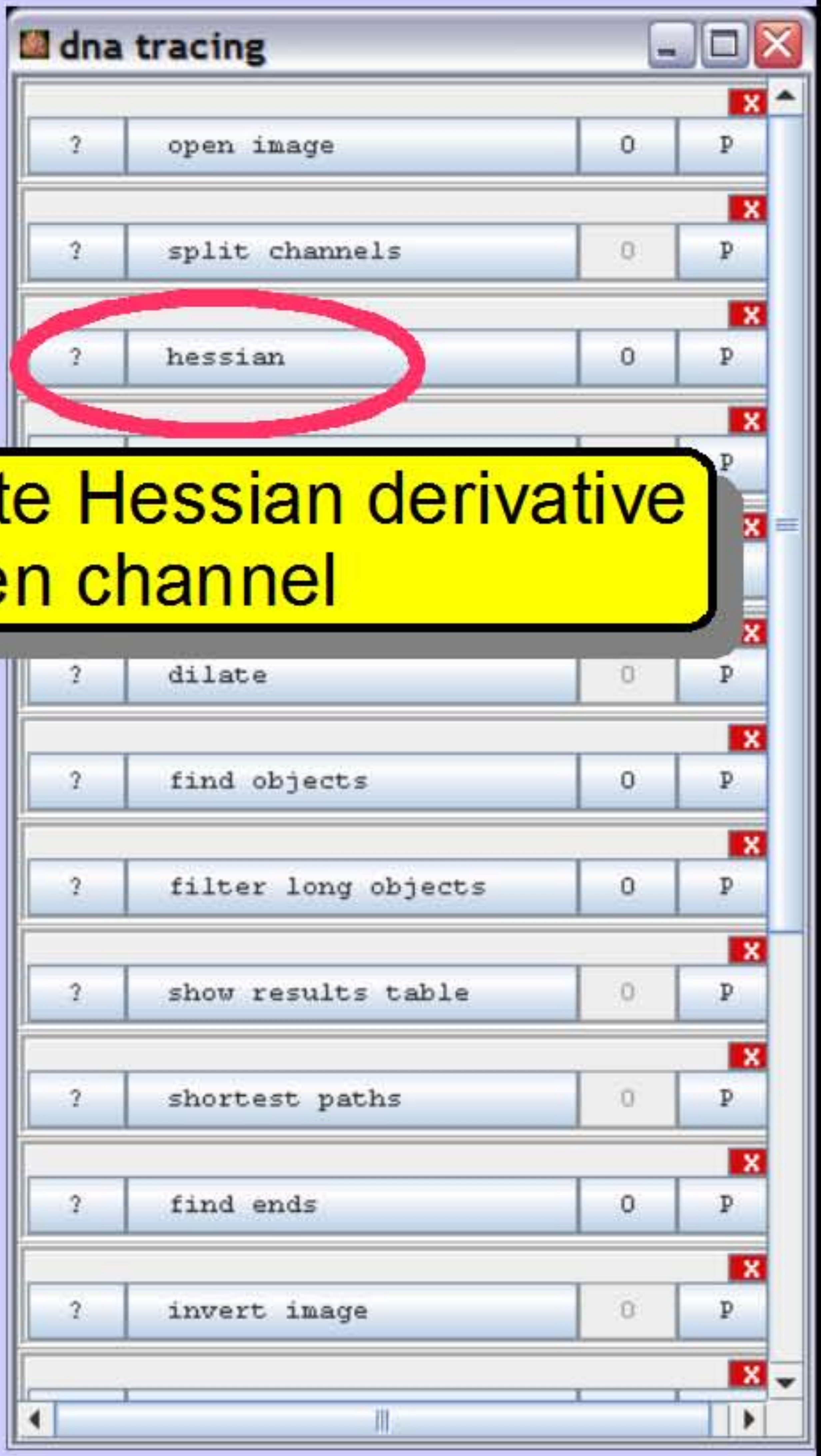


dna tracing batch

?	foreach image do	0	P
?	open image	0	P
?	split channels	0	P
?	hessian	0	P
?	get image from hessian	0	P
?	auto threshold	0	P
?	dilate	0	P
?	find objects	0	P
?	filter long objects	0	P
?	shortest paths	0	P
?	find ends	0	P
?	invert image	0	P

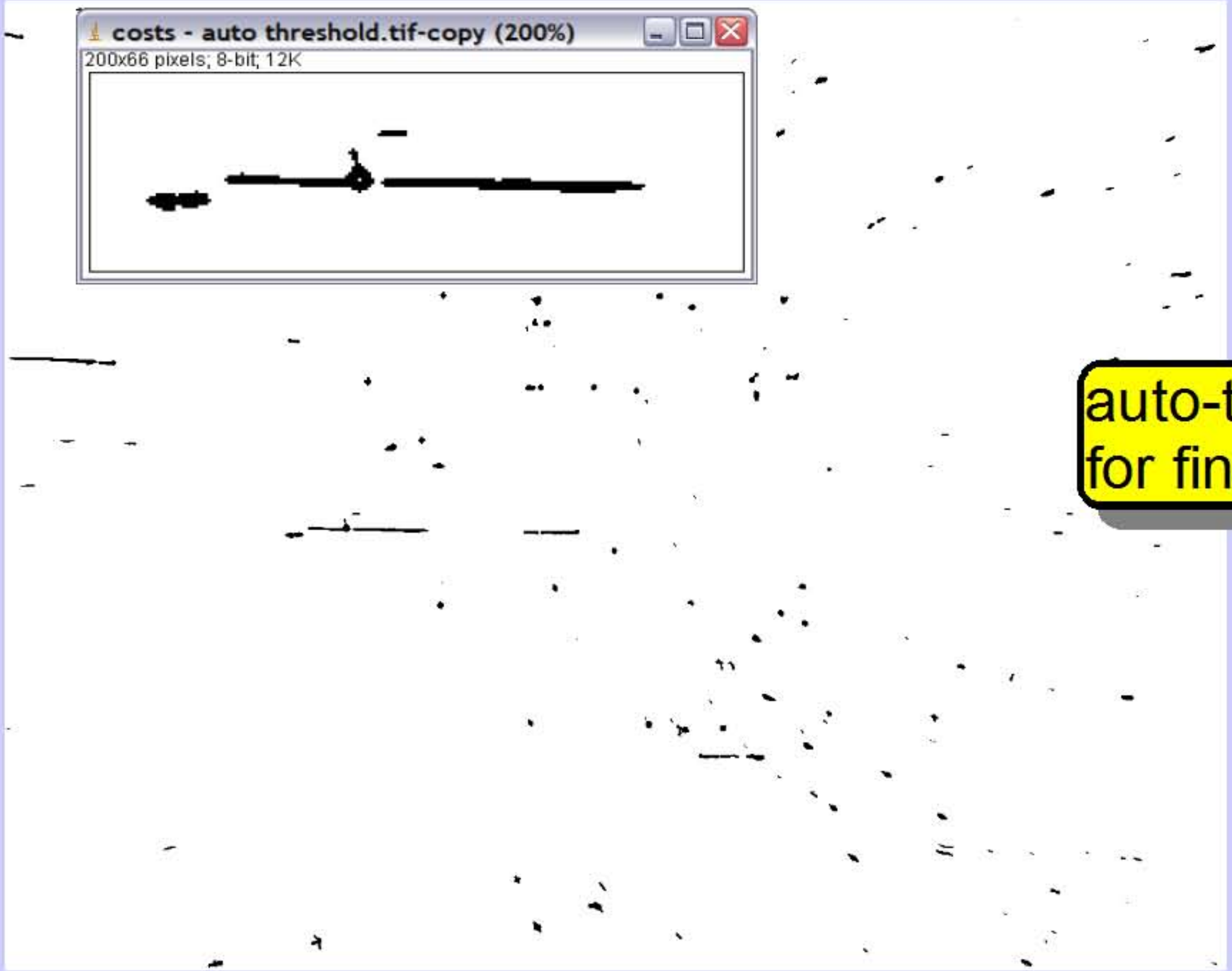
DNA combing





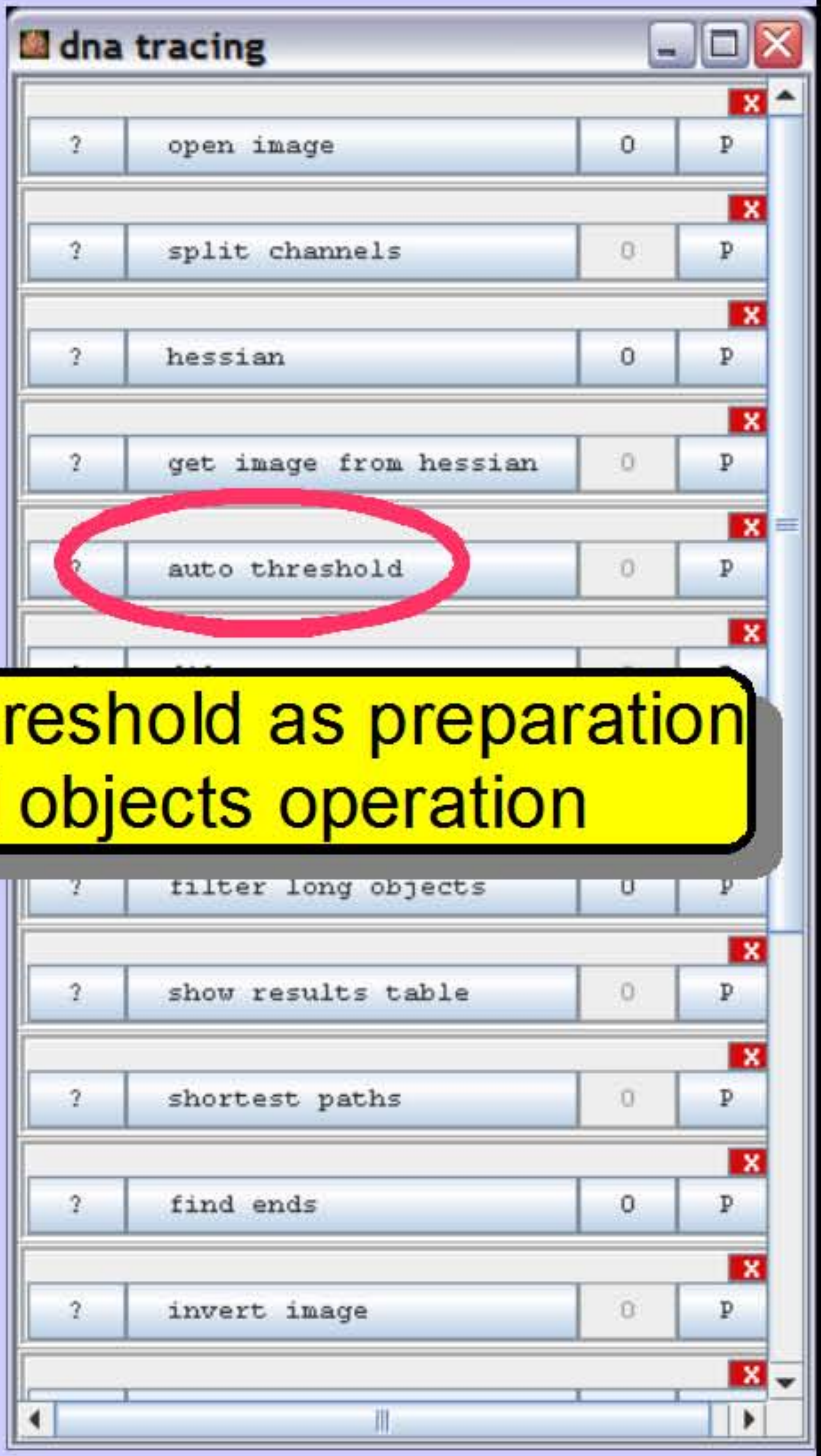
calculate Hessian derivative on green channel

DNA combing



costs - auto threshold.tif-copy (200%)

200x66 pixels; 8-bit; 12K

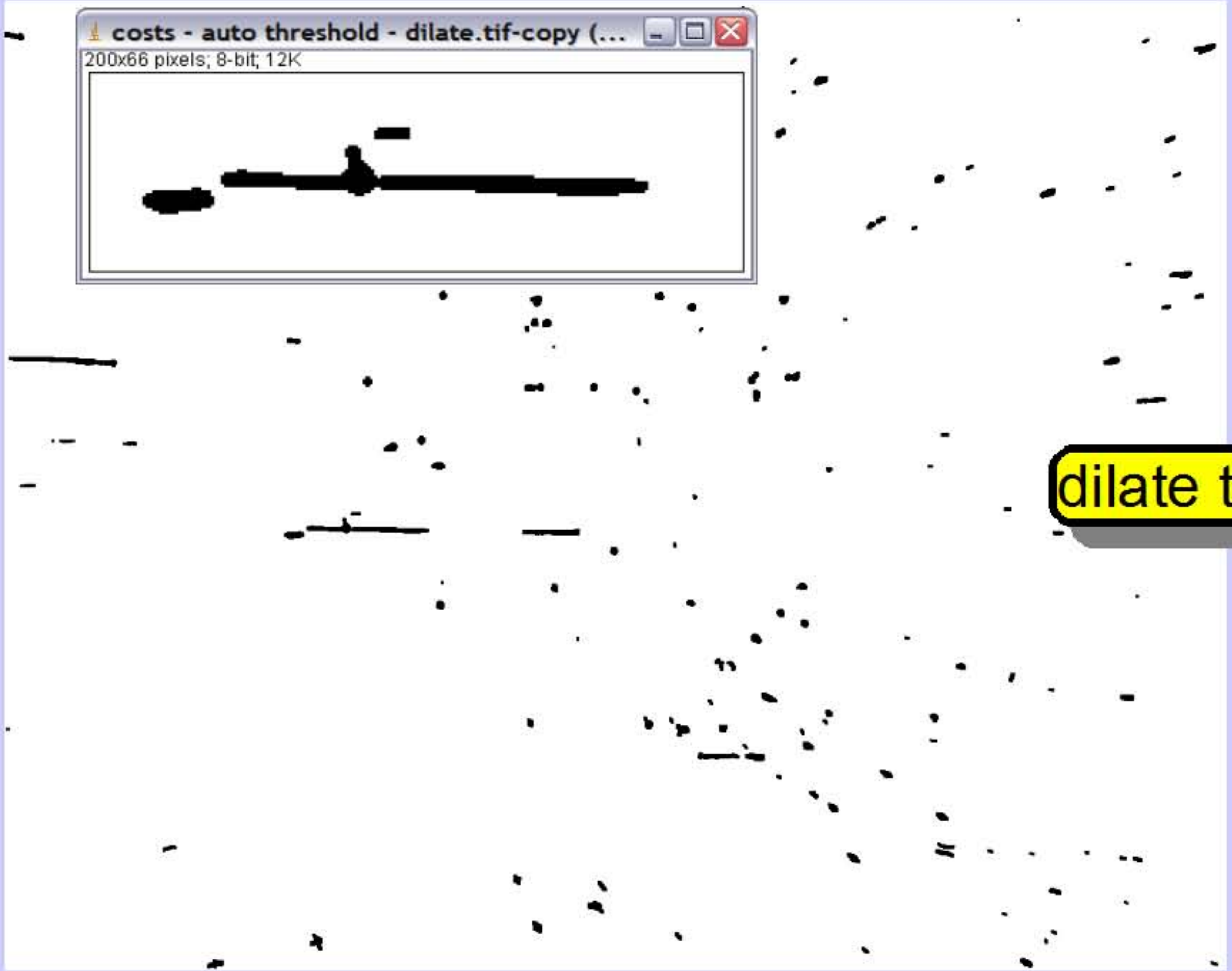


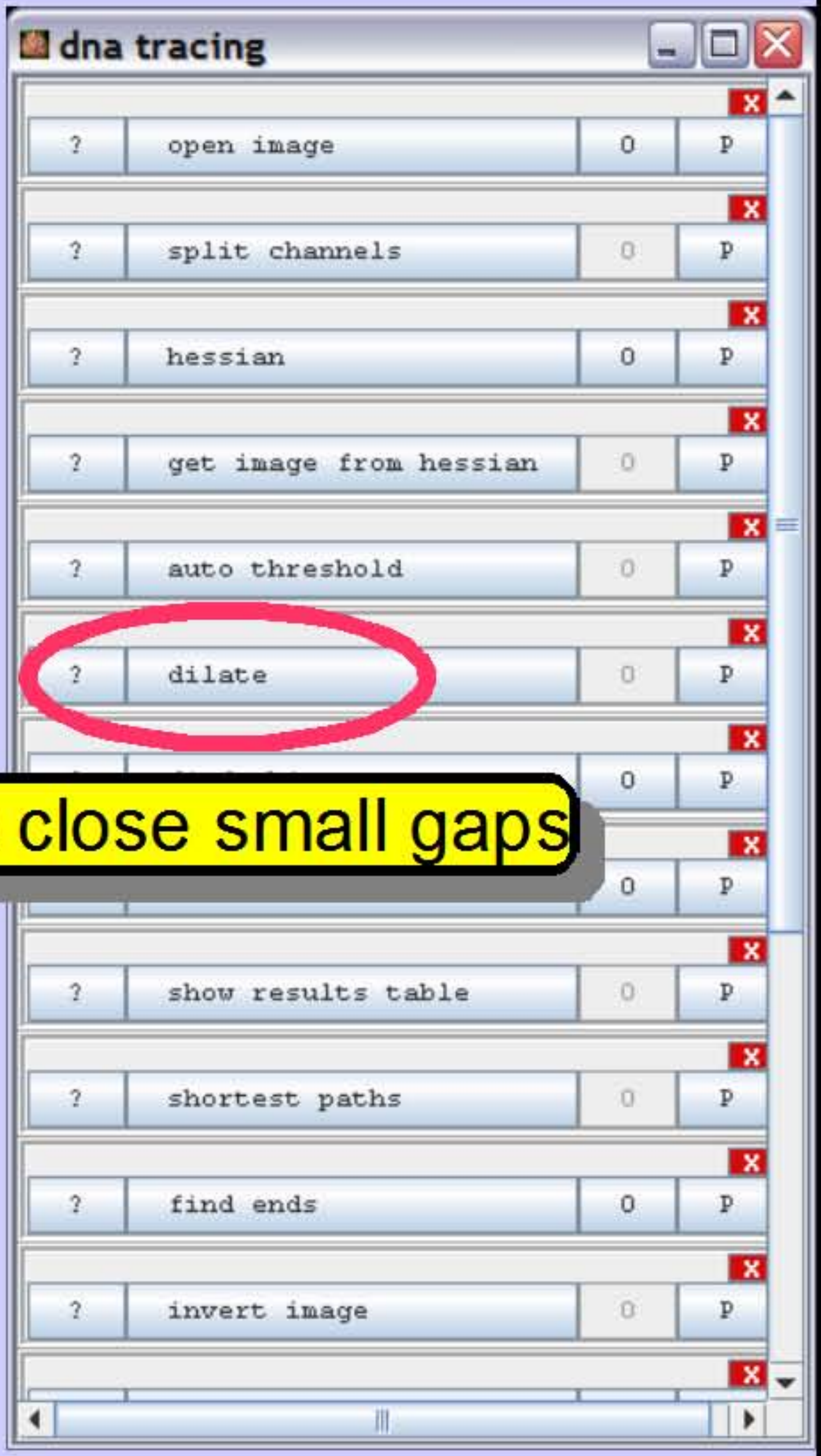
dna tracing

?	open image	0	P
?	split channels	0	P
?	hessian	0	P
?	get image from hessian	0	P
?	auto threshold	0	P
?	filter long objects	0	P
?	show results table	0	P
?	shortest paths	0	P
?	find ends	0	P
?	invert image	0	P

auto-threshold as preparation for find objects operation

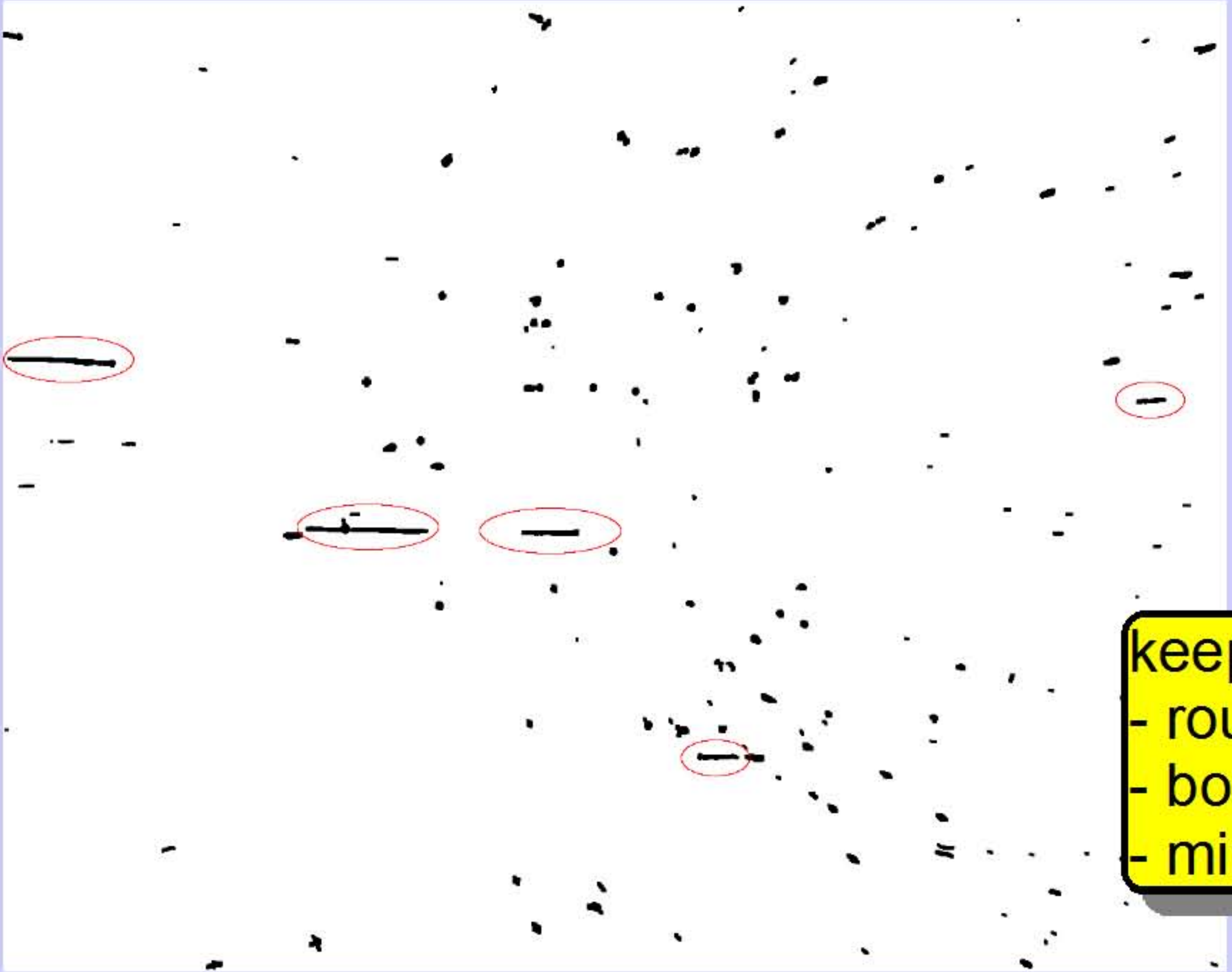
DNA combing

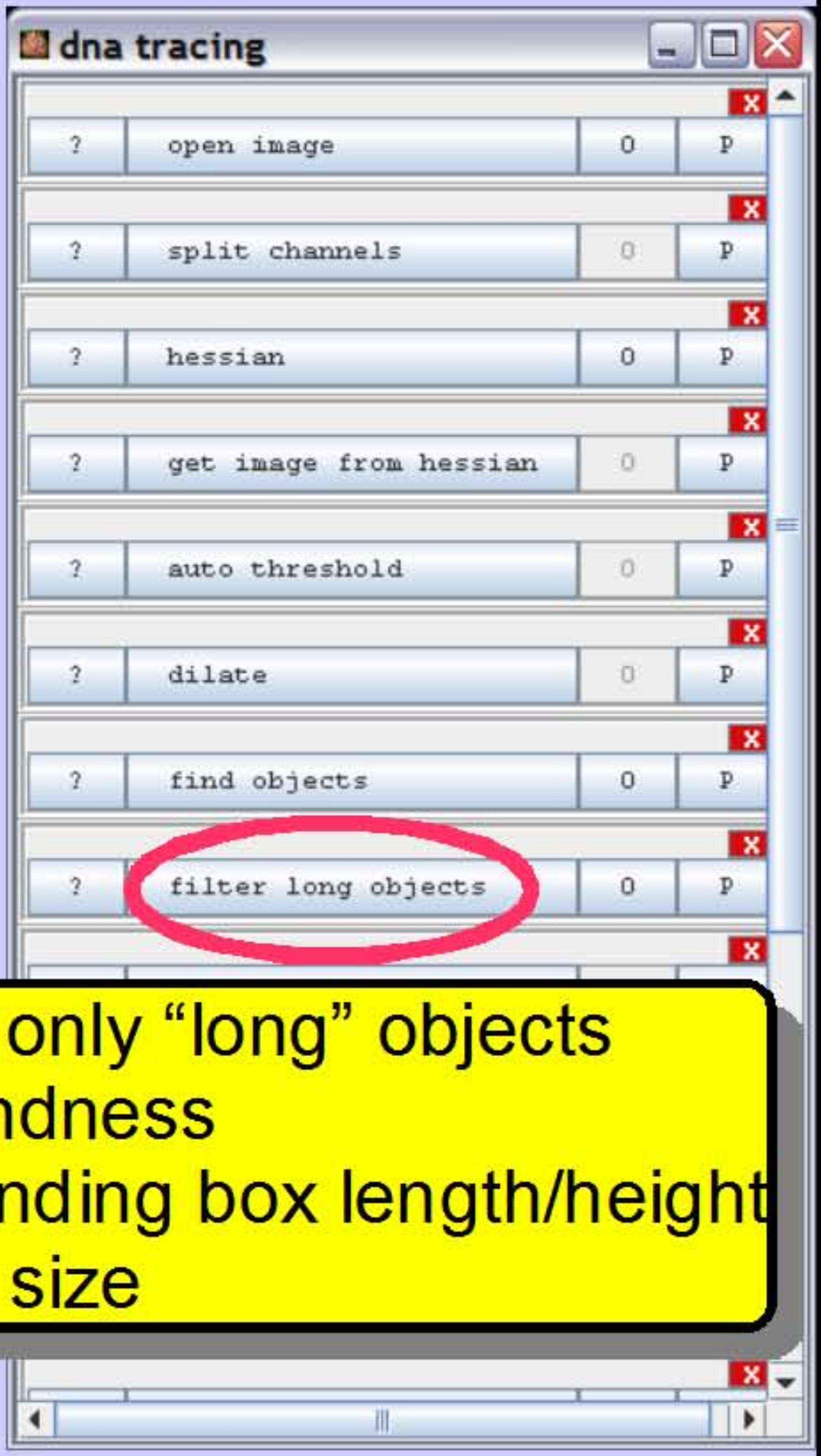

costs - auto threshold - dilate.tif-copy (...
200x66 pixels; 8-bit; 12K



dilate to close small gaps

DNA combing



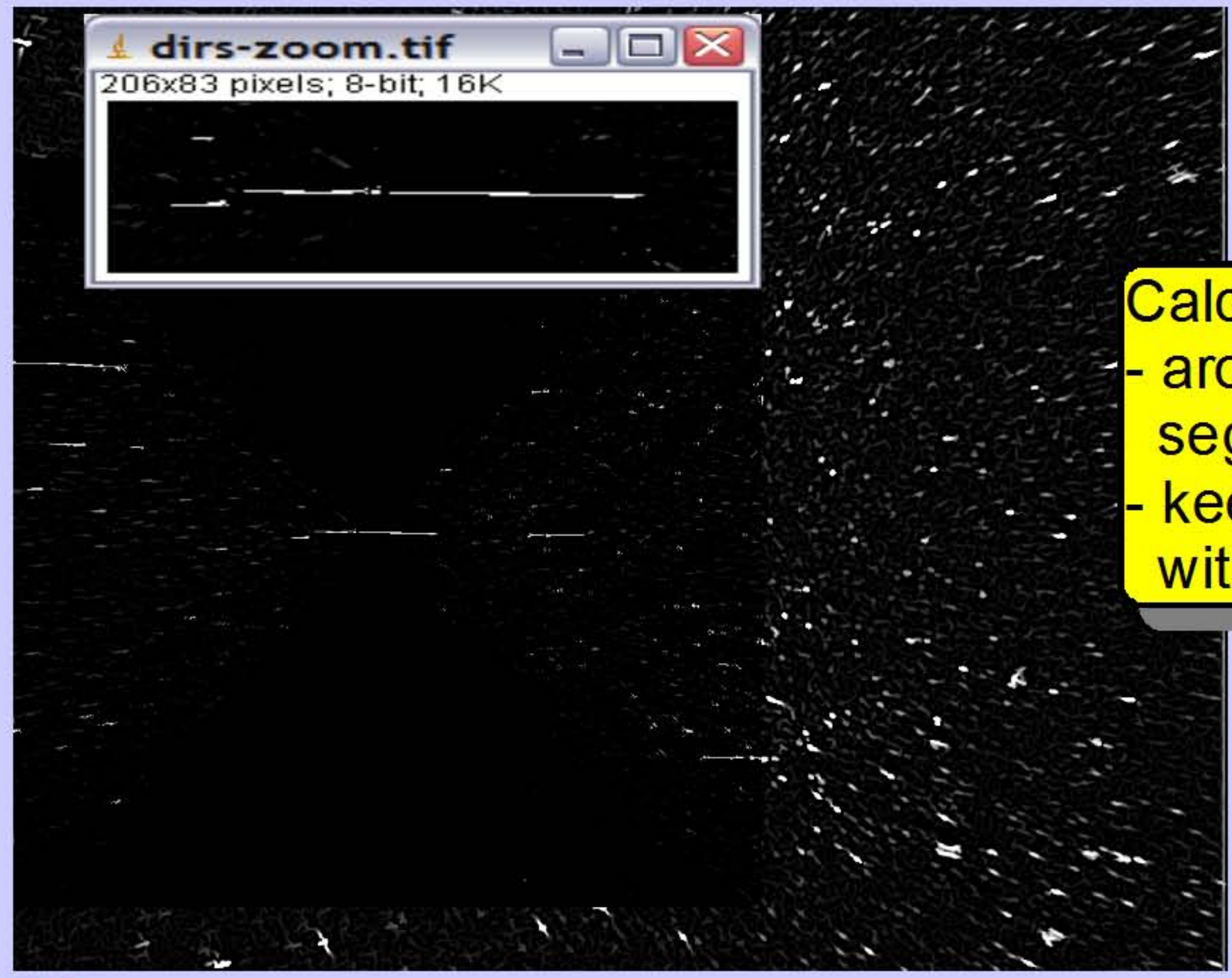


dna tracing

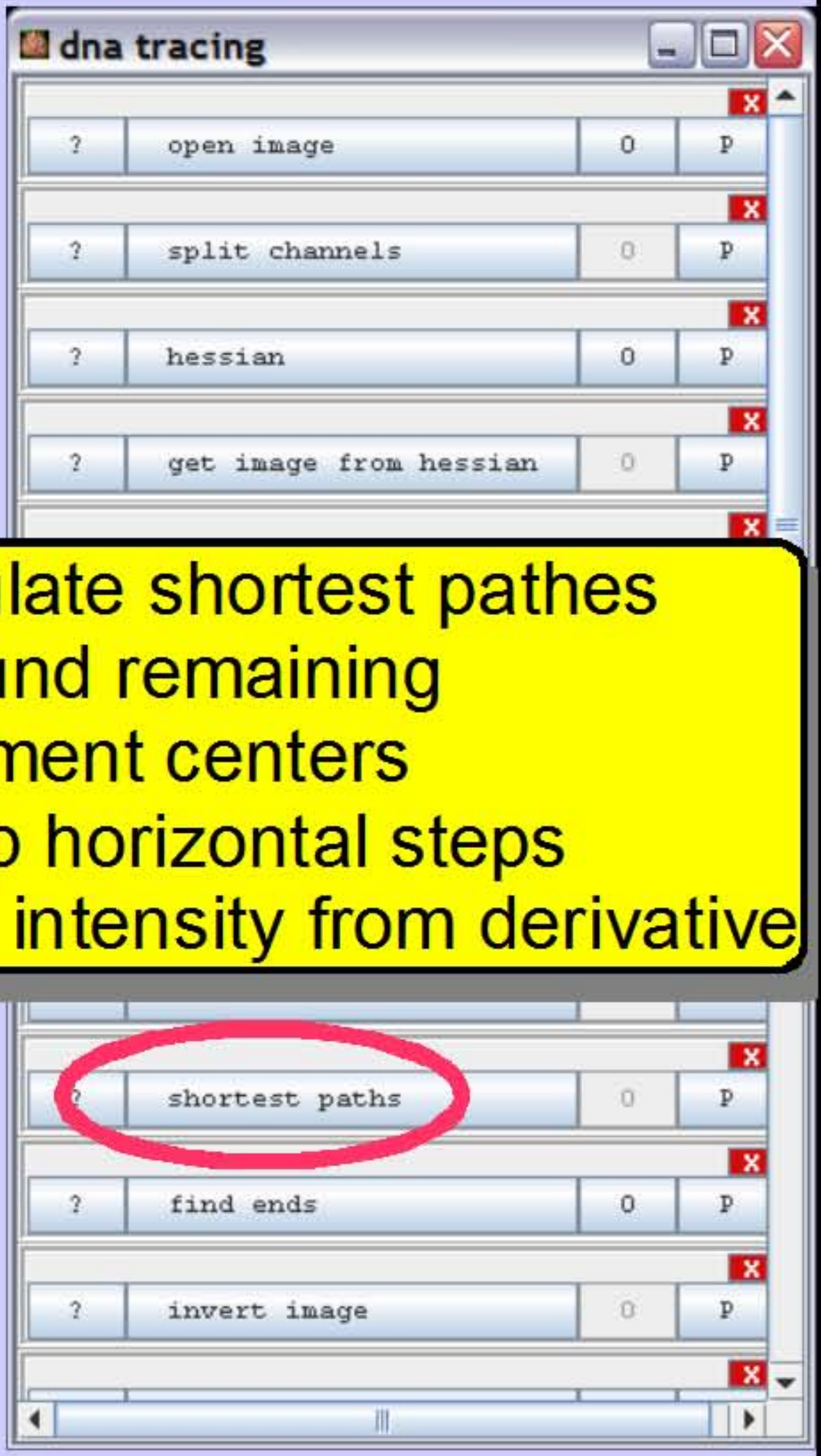
?	open image	0	P
?	split channels	0	P
?	hessian	0	P
?	get image from hessian	0	P
?	auto threshold	0	P
?	dilate	0	P
?	find objects	0	P
?	filter long objects	0	P

keep only “long” objects
- roundness
- bounding box length/height
- min size

DNA combing

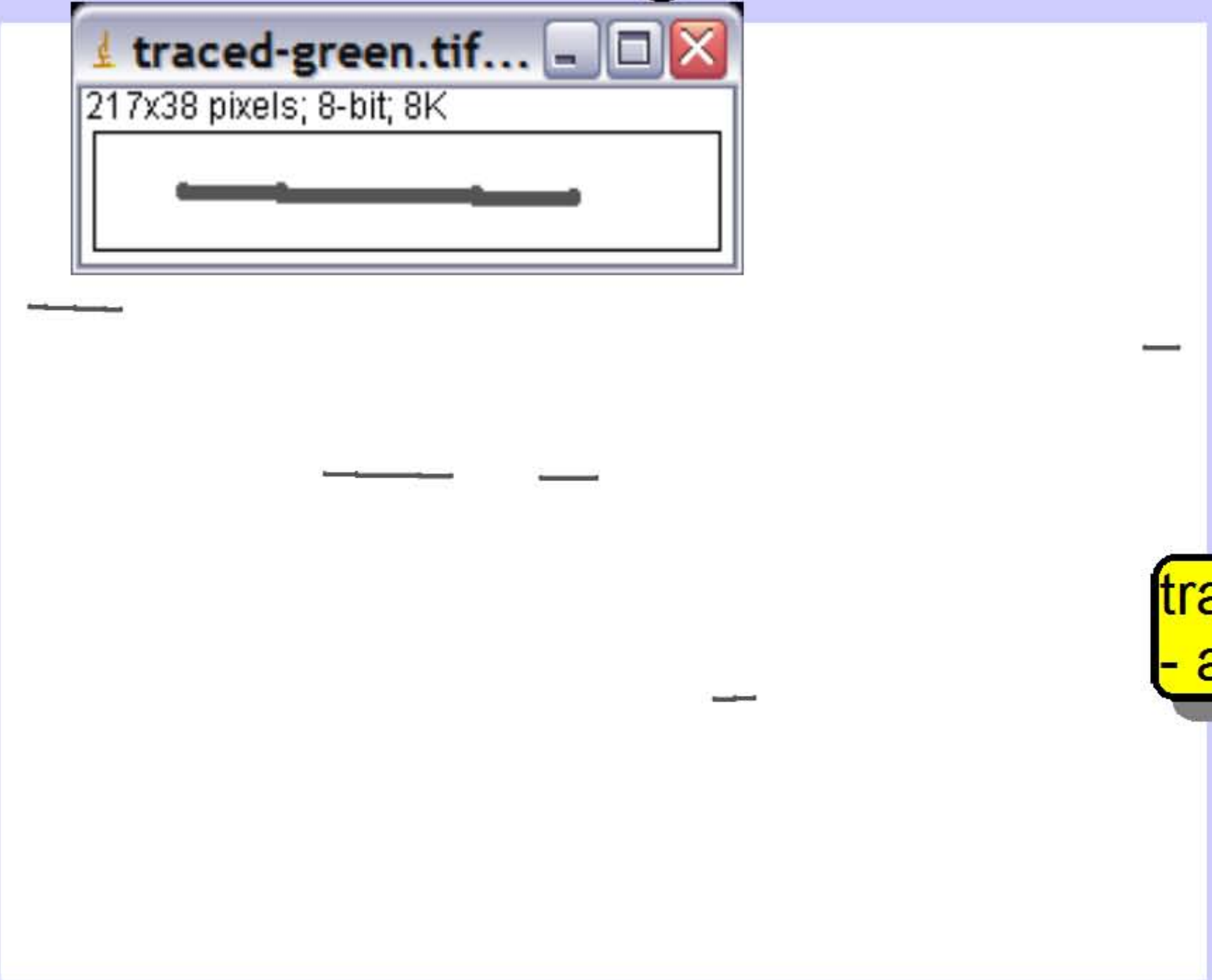


206x83 pixels; 8-bit; 16K

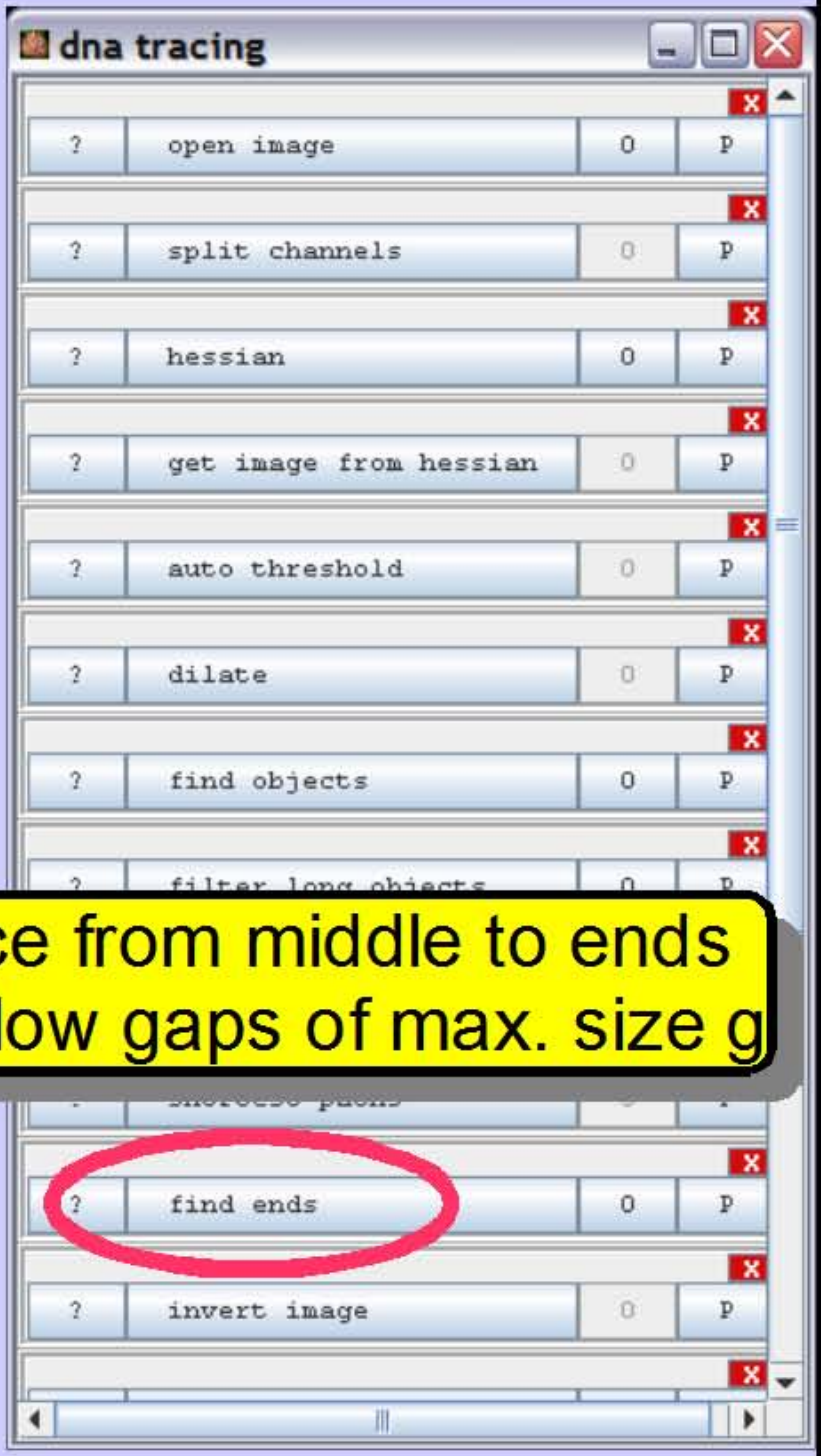


Calculate shortest pathes
- around remaining segment centers
- keep horizontal steps with intensity from derivative

DNA combing

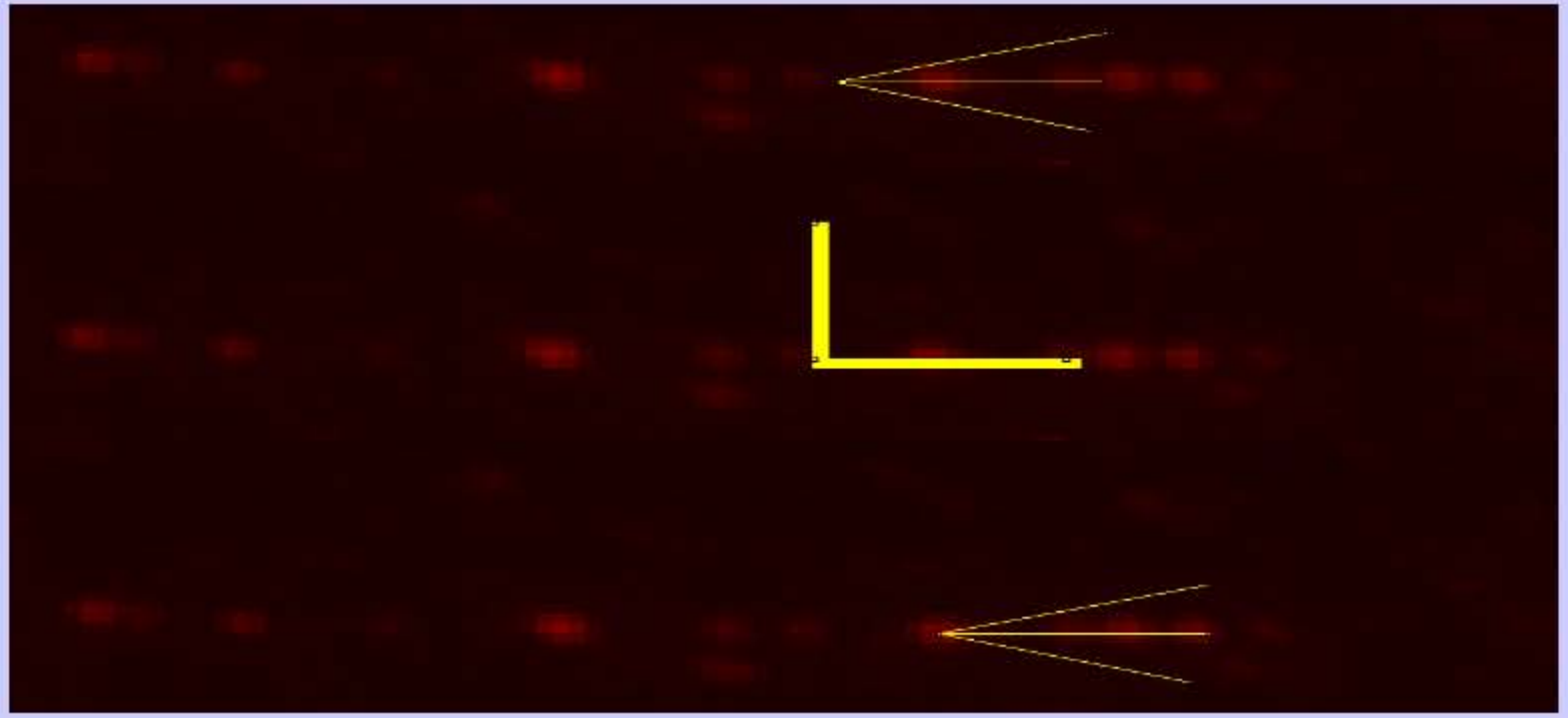


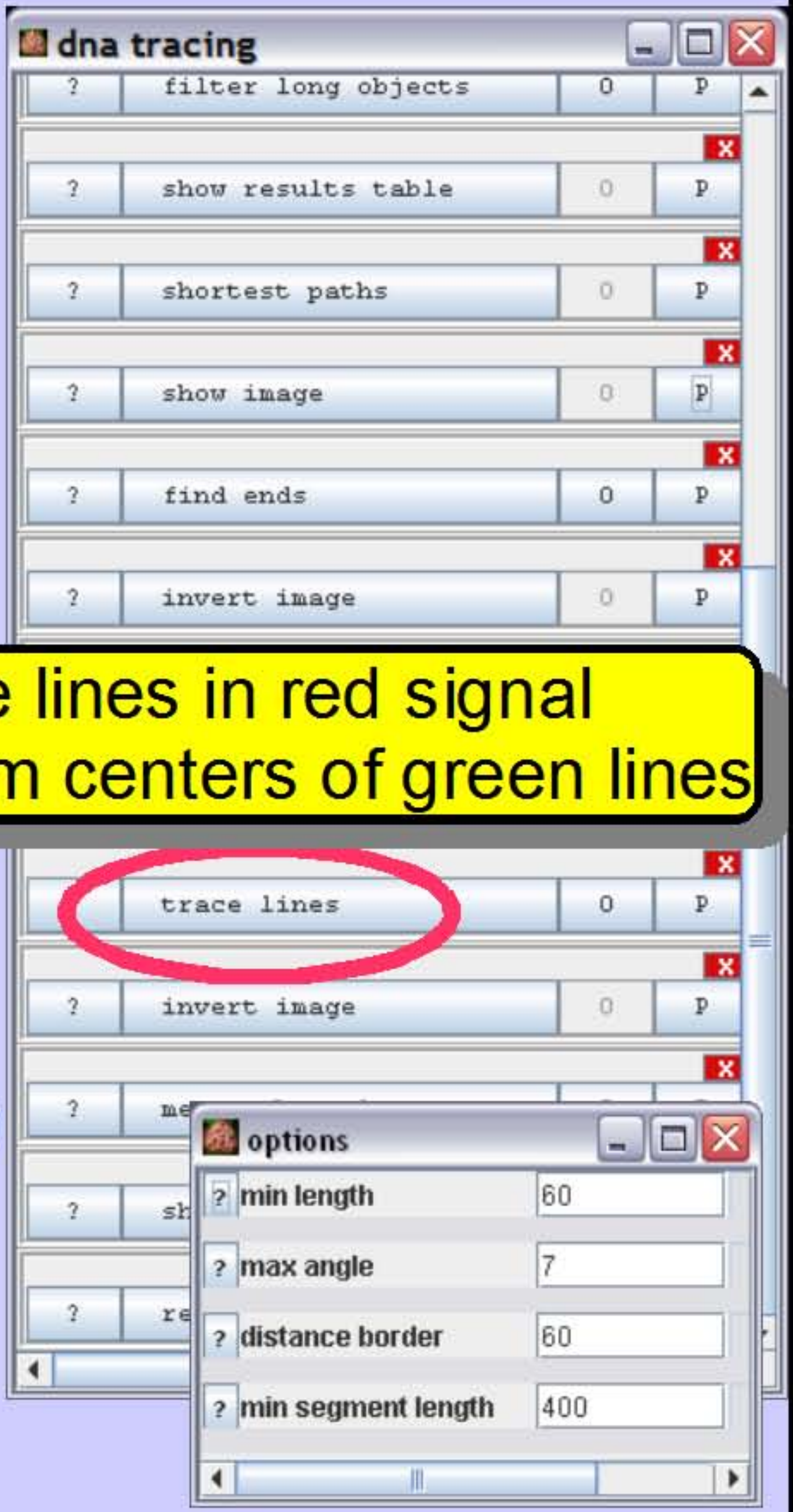
217x38 pixels; 8-bit; 8K



trace from middle to ends
- allow gaps of max. size g

DNA combing





dna tracing

?	filter long objects	0	P
?	show results table	0	P
?	shortest paths	0	P
?	show image	0	P
?	find ends	0	P
?	invert image	0	P
?	trace lines	0	P
?	invert image	0	P
?	me		
?	sh		
?	re		

options

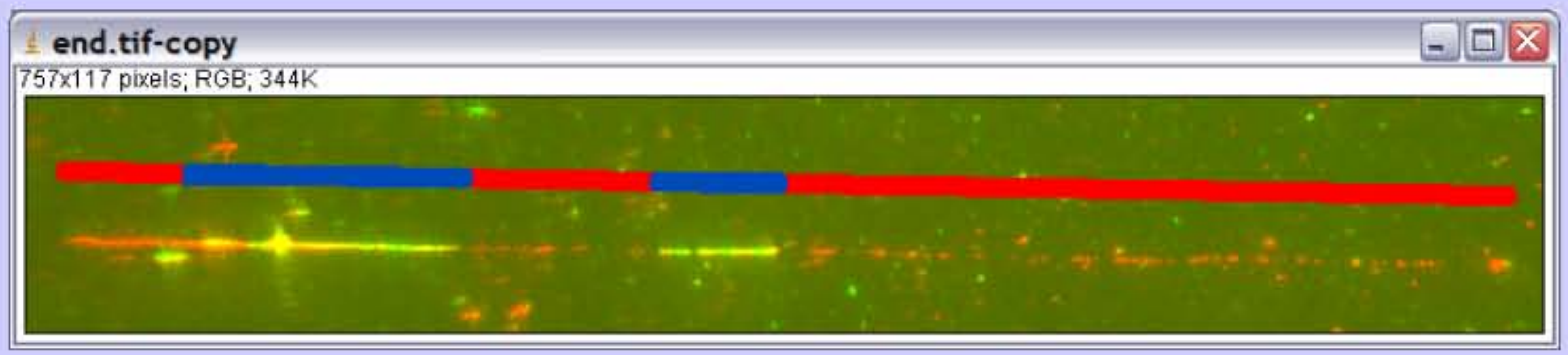
? min length	60
? max angle	7
? distance border	60
? min segment length	400

trace lines in red signal
- from centers of green lines

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

DNA combing



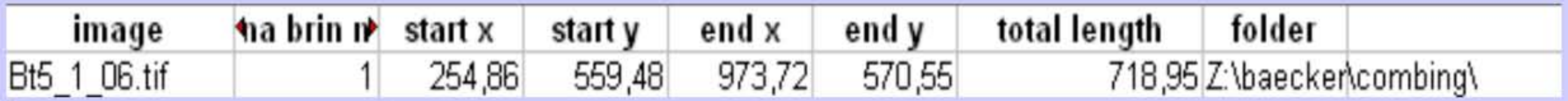
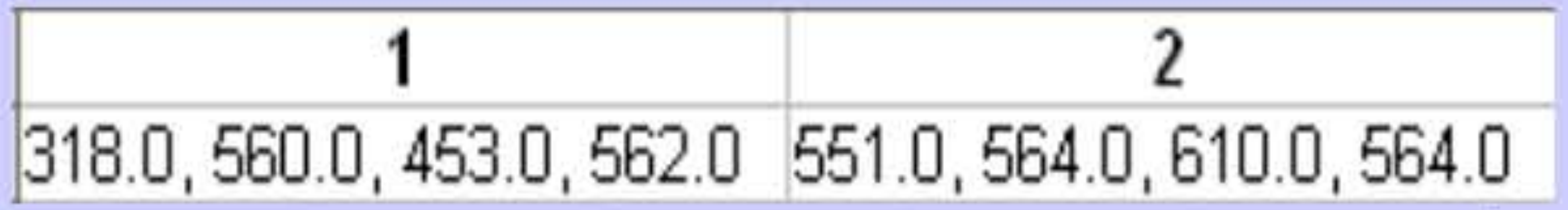
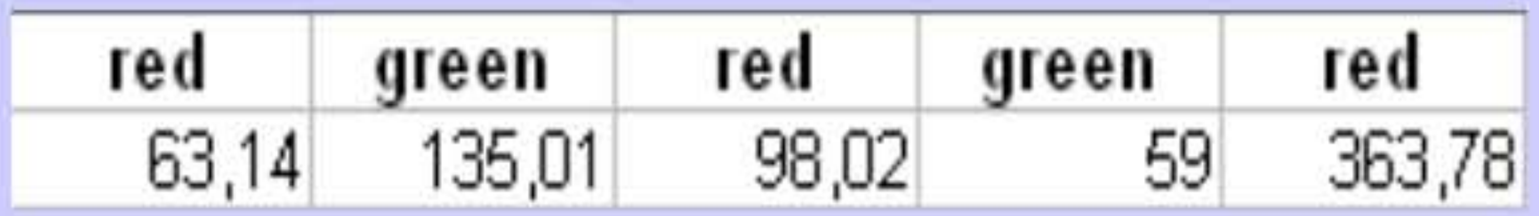


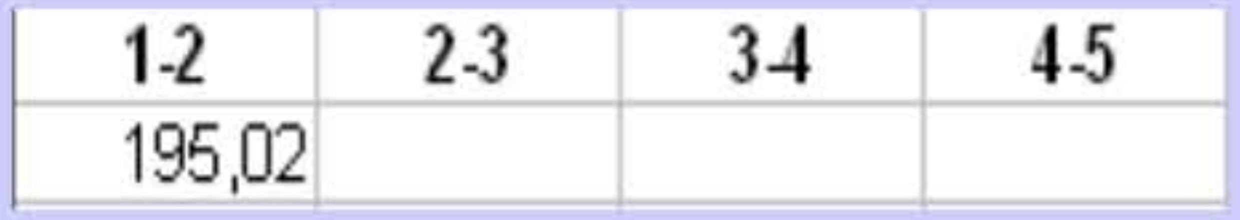
image	na brin n	start x	start y	end x	end y	total length	folder	
Bt5_1_06.tif	1	254,86	559,48	973,72	570,55	718,95	Z:\baecker\combing\	



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			

Report:
- total length
- length of each segment
- distances between centers of green segments

DNA combing, manual

use **slide show control** to select image

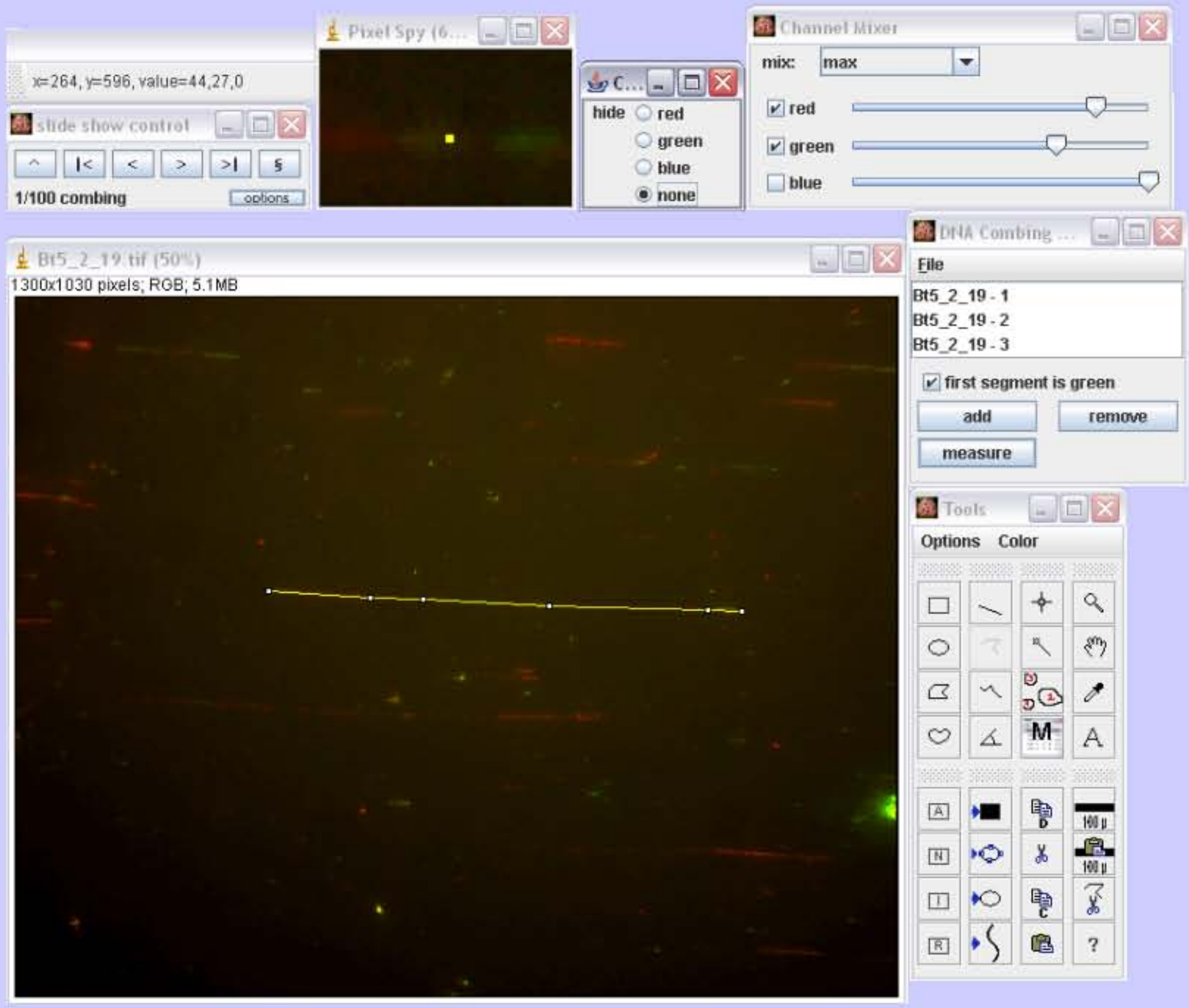
use **channel mixer** to adjust view

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



x=264, y=596, value=44,27,0

1/100 combing

hide ○ red ○ green ○ blue ● none

mix: max

☑ red

☑ green

□ blue

Bt5_2_19.tif (50%)

1300x1030 pixels; RGB; 5.1MB

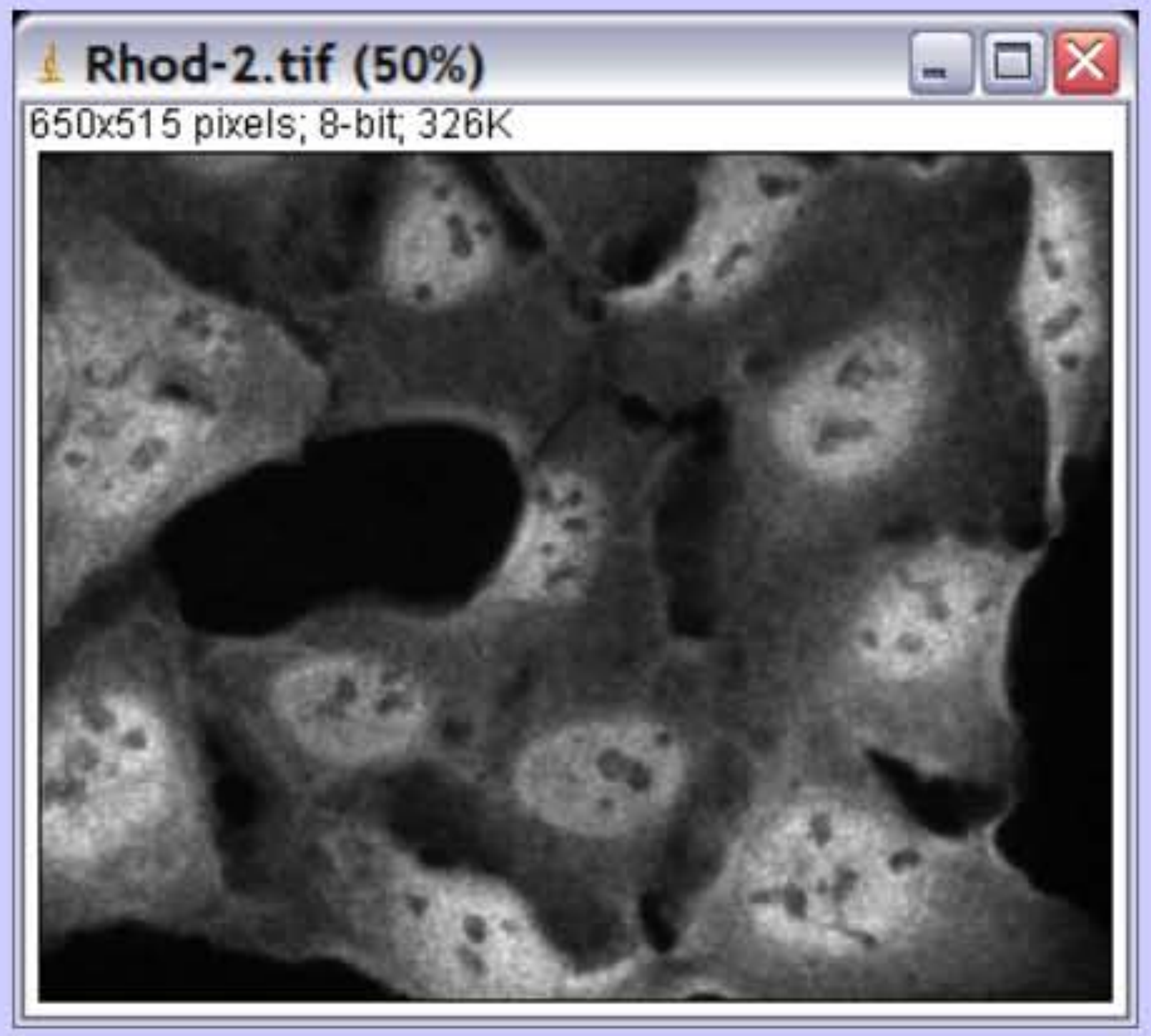
Bt5_2_19 - 1

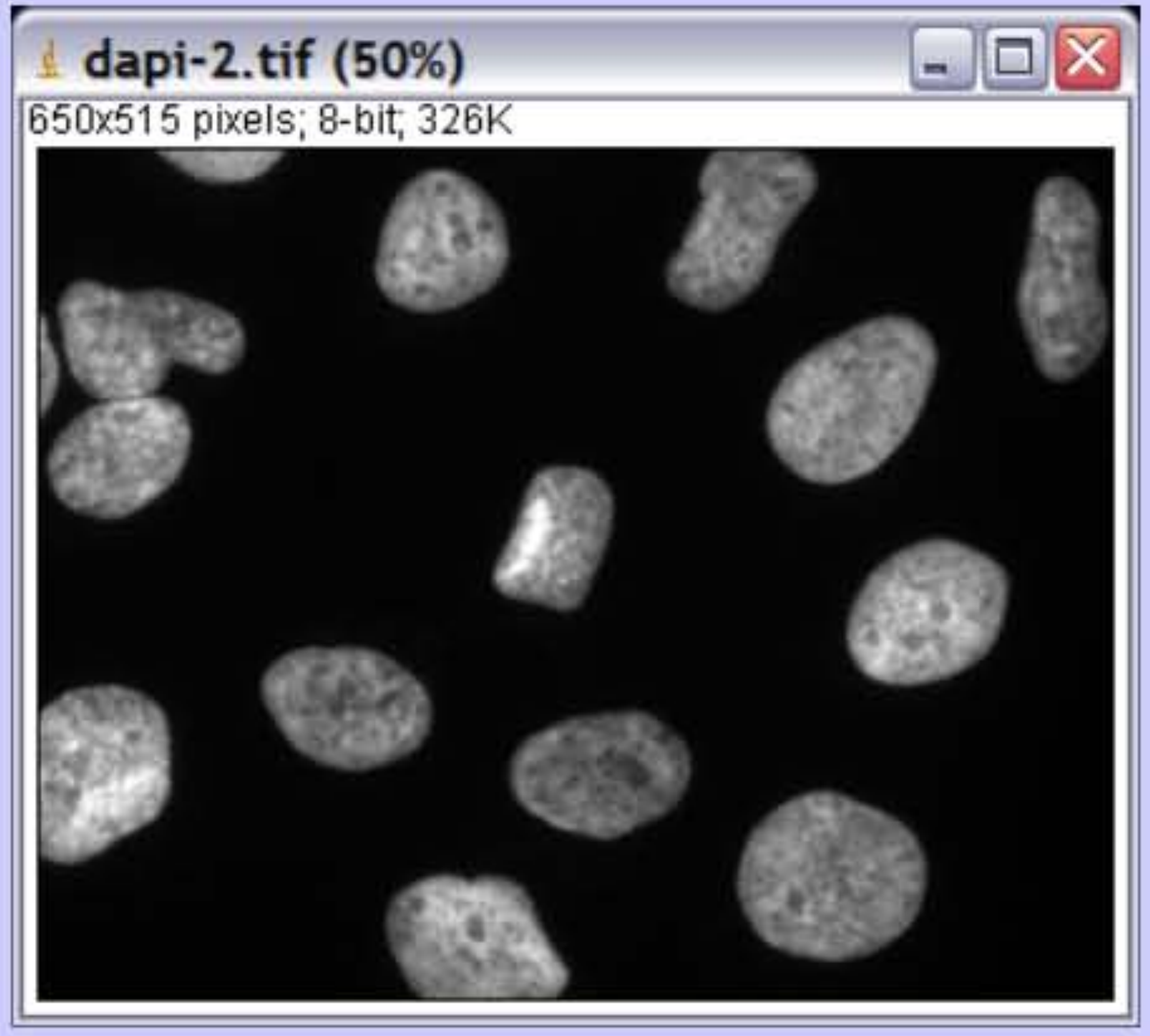
Bt5_2_19 - 2

Bt5_2_19 - 3

☑ first segment is green

Measuring intensity ratios

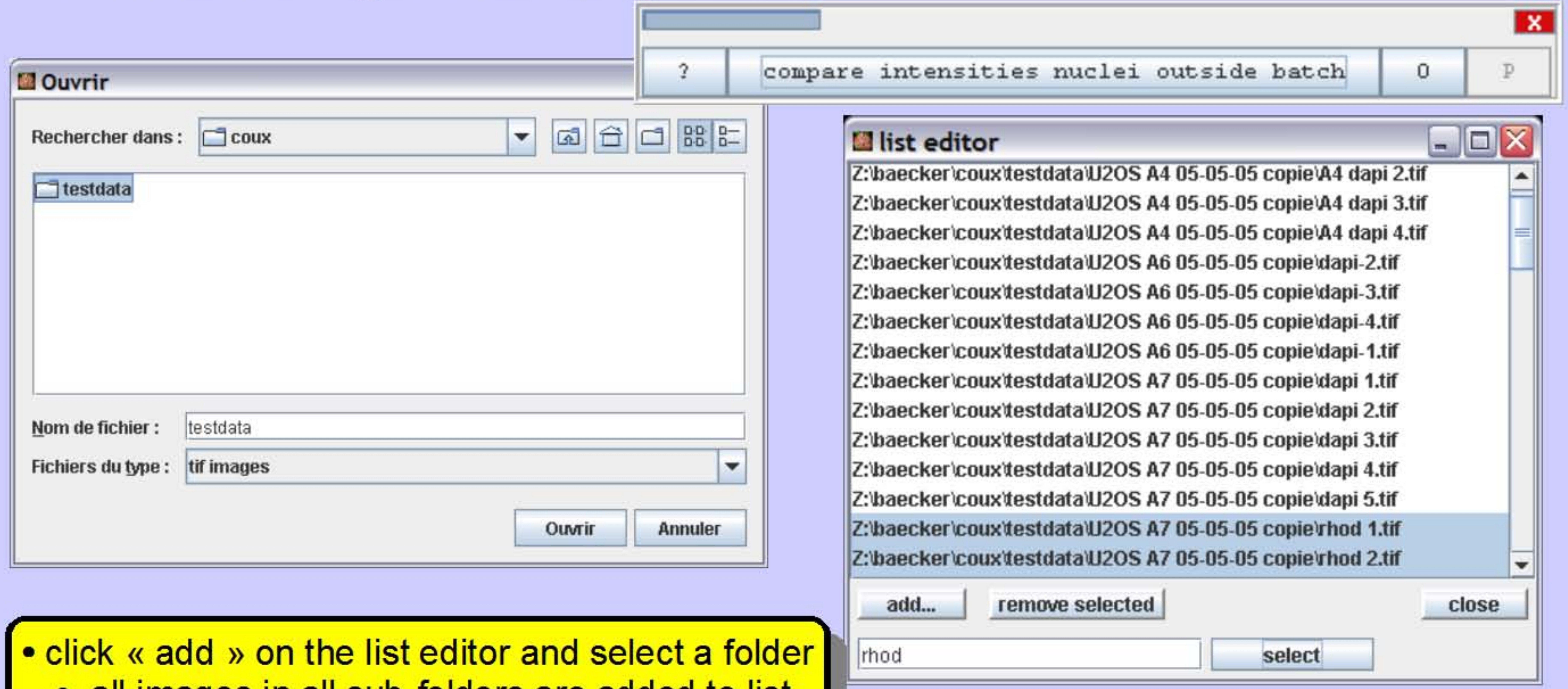




what is the proportion of fluorescence between nuclei and cytoplasm in the first image?

the second image is used to identify the nuclei

Measuring intensity ratios

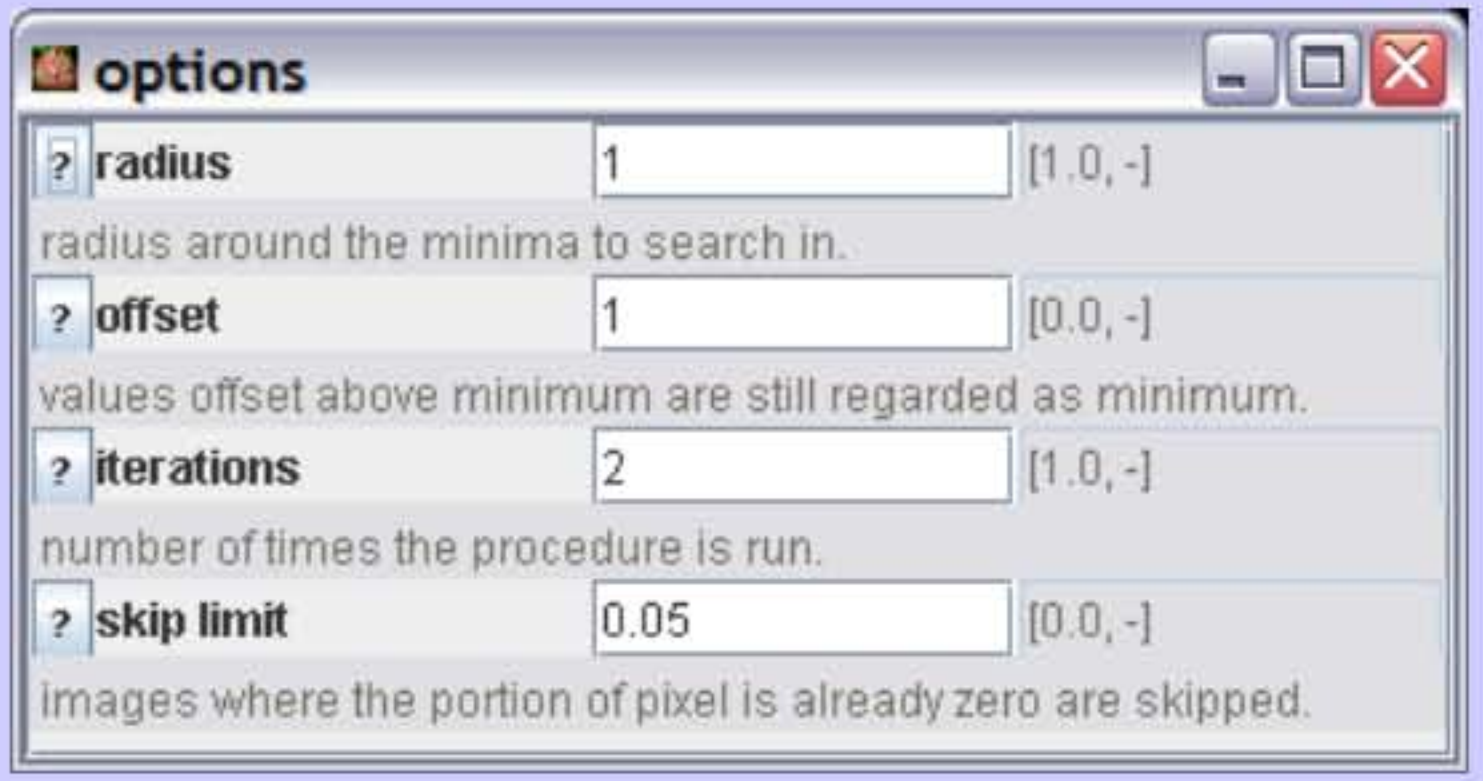


```
Z:\baecker\coux\testdata\U2OS A4 05-05-05 copie\A4 dapi 2.tif
Z:\baecker\coux\testdata\U2OS A4 05-05-05 copie\A4 dapi 3.tif
Z:\baecker\coux\testdata\U2OS A4 05-05-05 copie\A4 dapi 4.tif
Z:\baecker\coux\testdata\U2OS A6 05-05-05 copie\dapi-2.tif
Z:\baecker\coux\testdata\U2OS A6 05-05-05 copie\dapi-3.tif
Z:\baecker\coux\testdata\U2OS A6 05-05-05 copie\dapi-4.tif
Z:\baecker\coux\testdata\U2OS A6 05-05-05 copie\dapi-1.tif
Z:\baecker\coux\testdata\U2OS A7 05-05-05 copie\dapi 1.tif
Z:\baecker\coux\testdata\U2OS A7 05-05-05 copie\dapi 2.tif
Z:\baecker\coux\testdata\U2OS A7 05-05-05 copie\dapi 3.tif
Z:\baecker\coux\testdata\U2OS A7 05-05-05 copie\dapi 4.tif
Z:\baecker\coux\testdata\U2OS A7 05-05-05 copie\dapi 5.tif
Z:\baecker\coux\testdata\U2OS A7 05-05-05 copie\rhod 1.tif
Z:\baecker\coux\testdata\U2OS A7 05-05-05 copie\rhod 2.tif
```

- click « add » on the list editor and select a folder
  - all images in all sub-folders are added to list
  - use list editor to remove unwanted images

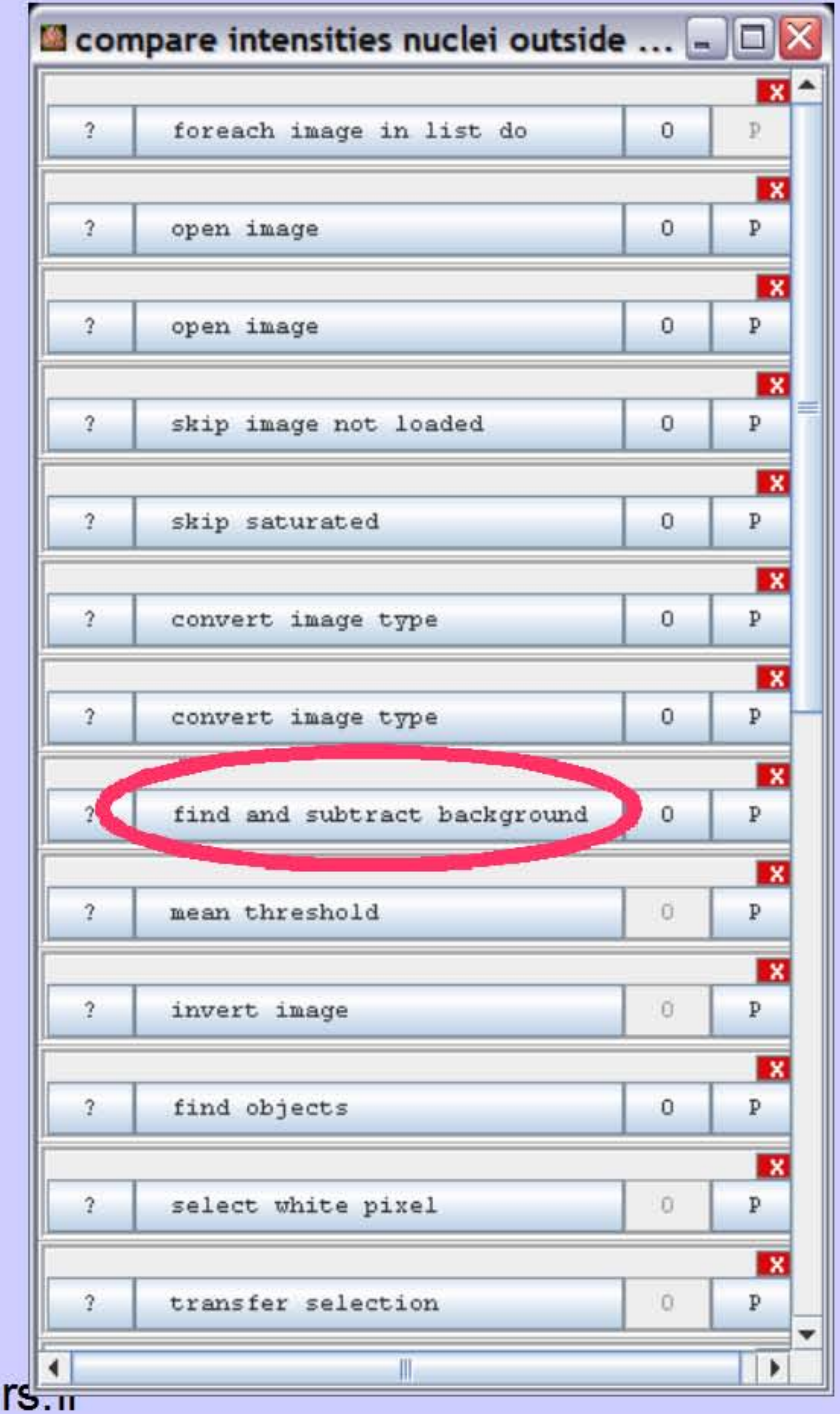
Measuring intensity ratios

- find and subtract background
  - find pixels P with intensity in [min, offset]
  - Find maximum intensity in area of size radius around pixels in P
  - subtract the max value from the image



options

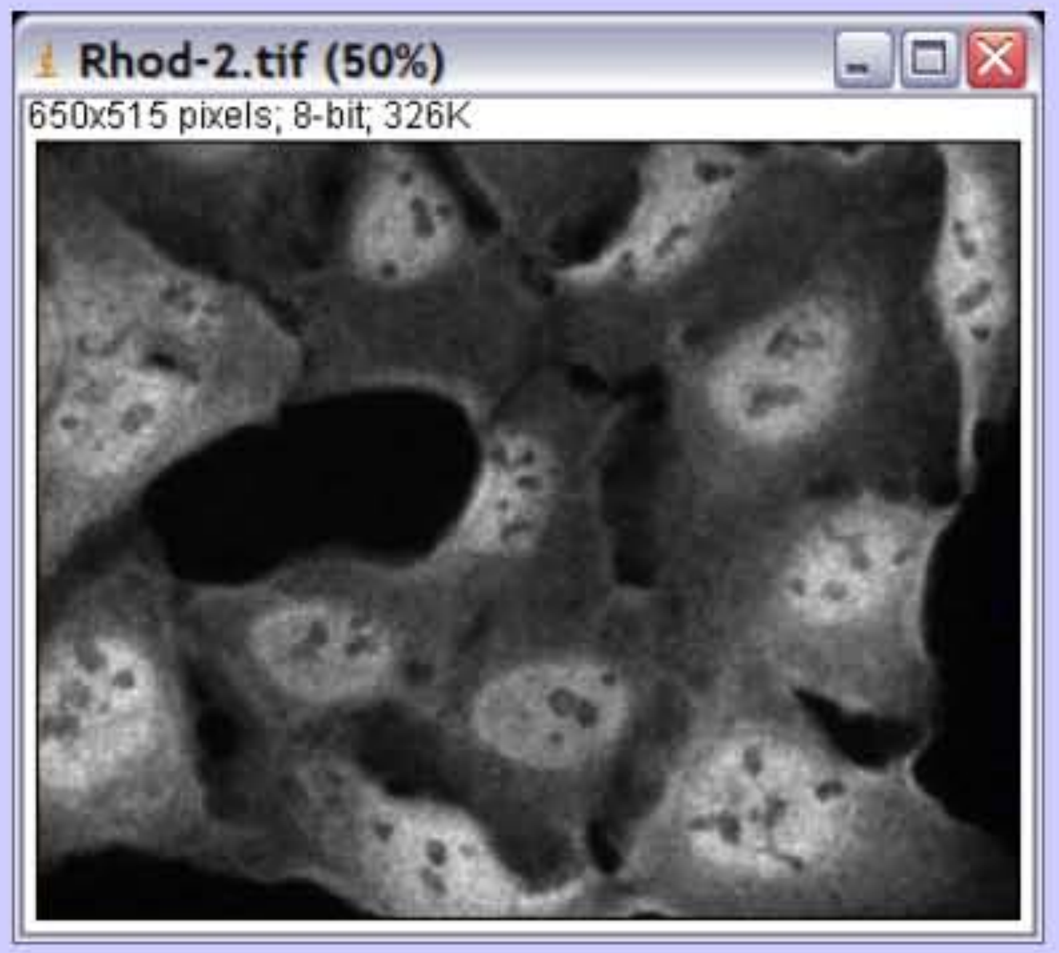
? radius	1	[1.0, -]
radius around the minima to search in.		
? offset	1	[0.0, -]
values offset above minimum are still regarded as minimum.		
? iterations	2	[1.0, -]
number of times the procedure is run.		
? skip limit	0.05	[0.0, -]
images where the portion of pixel is already zero are skipped.		



compare intensities nuclei outside ...

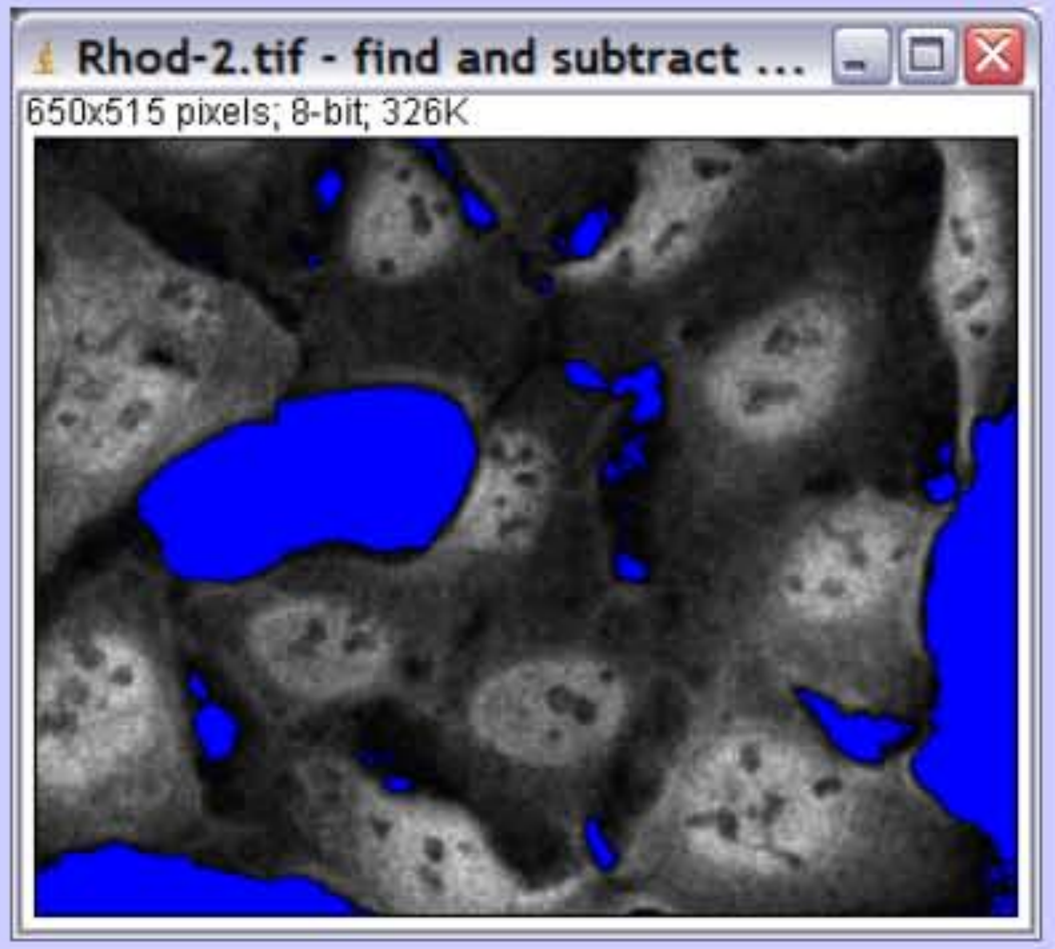
```
?  foreach image in list do        0  P
?  open image                      0  P
?  open image                      0  P
?  skip image not loaded           0  P
?  skip saturated                  0  P
?  convert image type              0  P
?  convert image type              0  P
?  find and subtract background    0  P
?  mean threshold                  0  P
?  invert image                    0  P
?  find objects                    0  P
?  select white pixel              0  P
?  transfer selection              0  P
```

Measuring intensity ratios



Rhod-2.tif (50%)

650x515 pixels; 8-bit; 326K

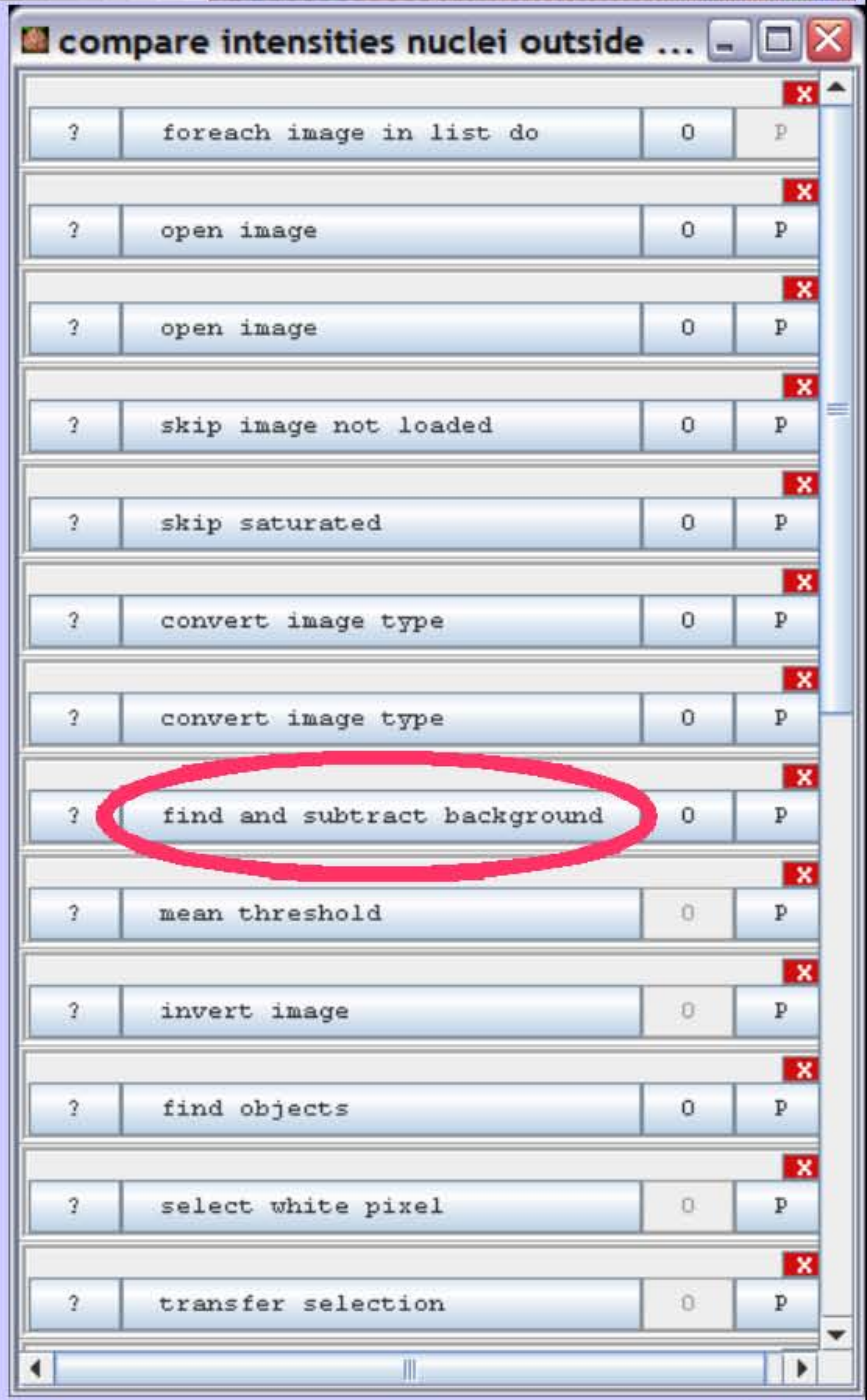


Rhod-2.tif - find and subtract ...

650x515 pixels; 8-bit; 326K

both images with hilo LUT
after background subtraction

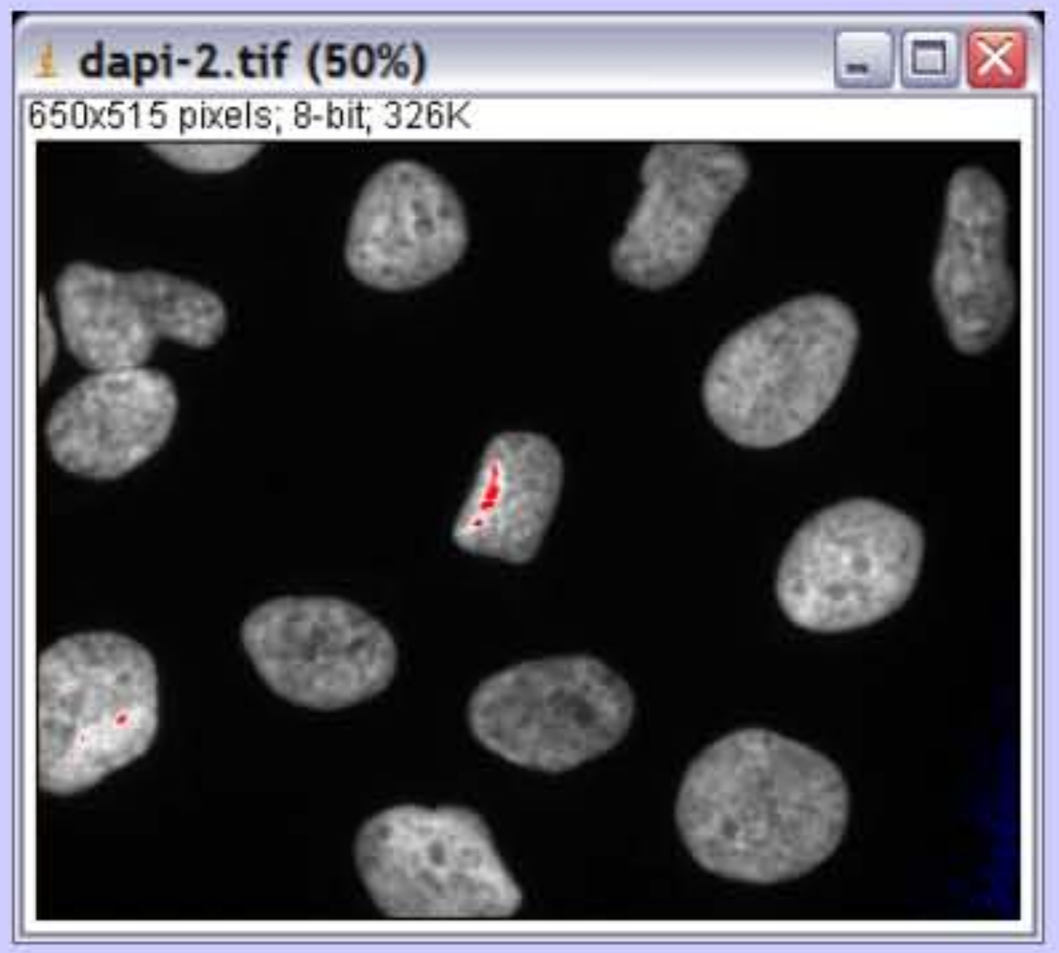
blue = intensity is zero



compare intensities nuclei outside ...

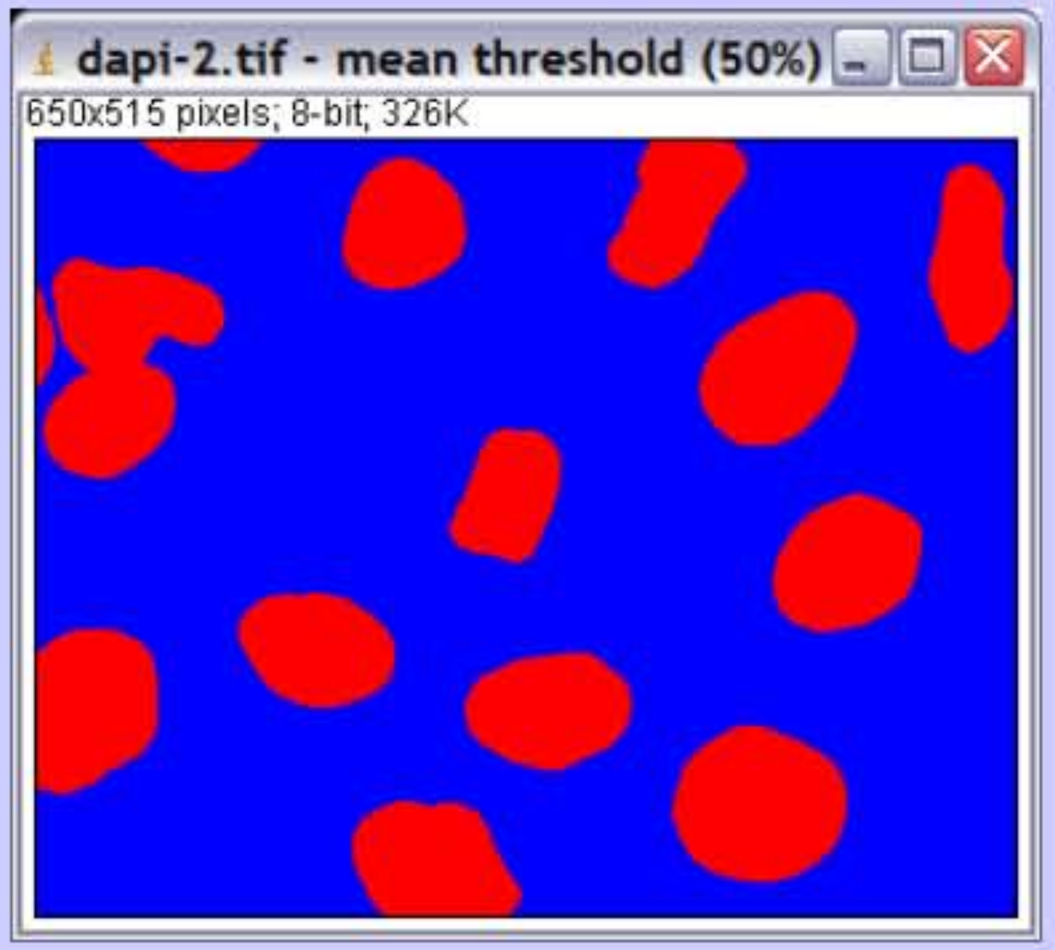
```
?  foreach image in list do     0  P
?  open image                   0  P
?  open image                   0  P
?  skip image not loaded        0  P
?  skip saturated               0  P
?  convert image type           0  P
?  convert image type           0  P
?  find and subtract background 0  P
?  mean threshold               0  P
?  invert image                 0  P
?  find objects                 0  P
?  select white pixel           0  P
?  transfer selection           0  P
```

Measuring intensity ratios



dapi-2.tif (50%)

650x515 pixels; 8-bit; 326K

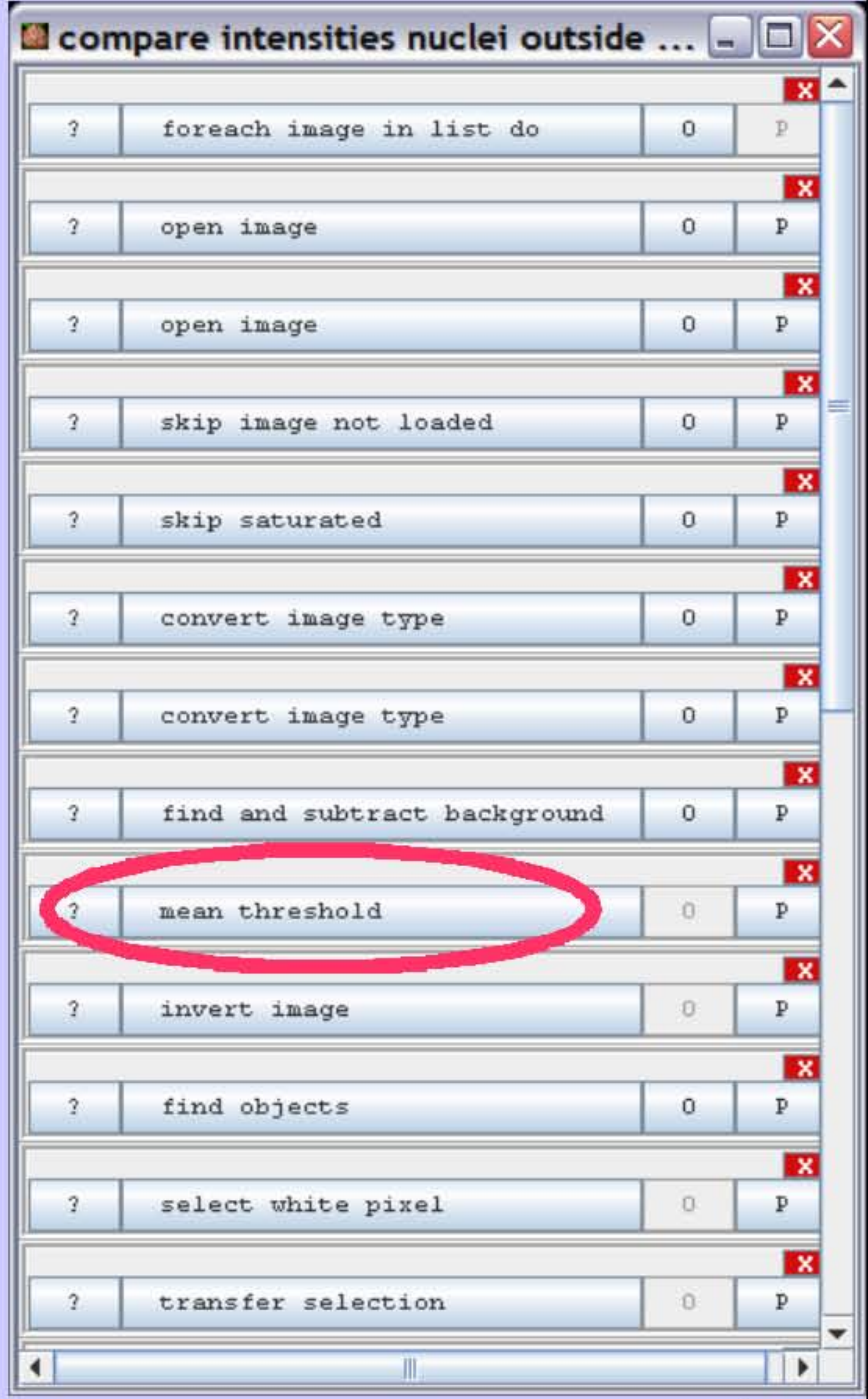


dapi-2.tif - mean threshold (50%)

650x515 pixels; 8-bit; 326K

to find nuclei

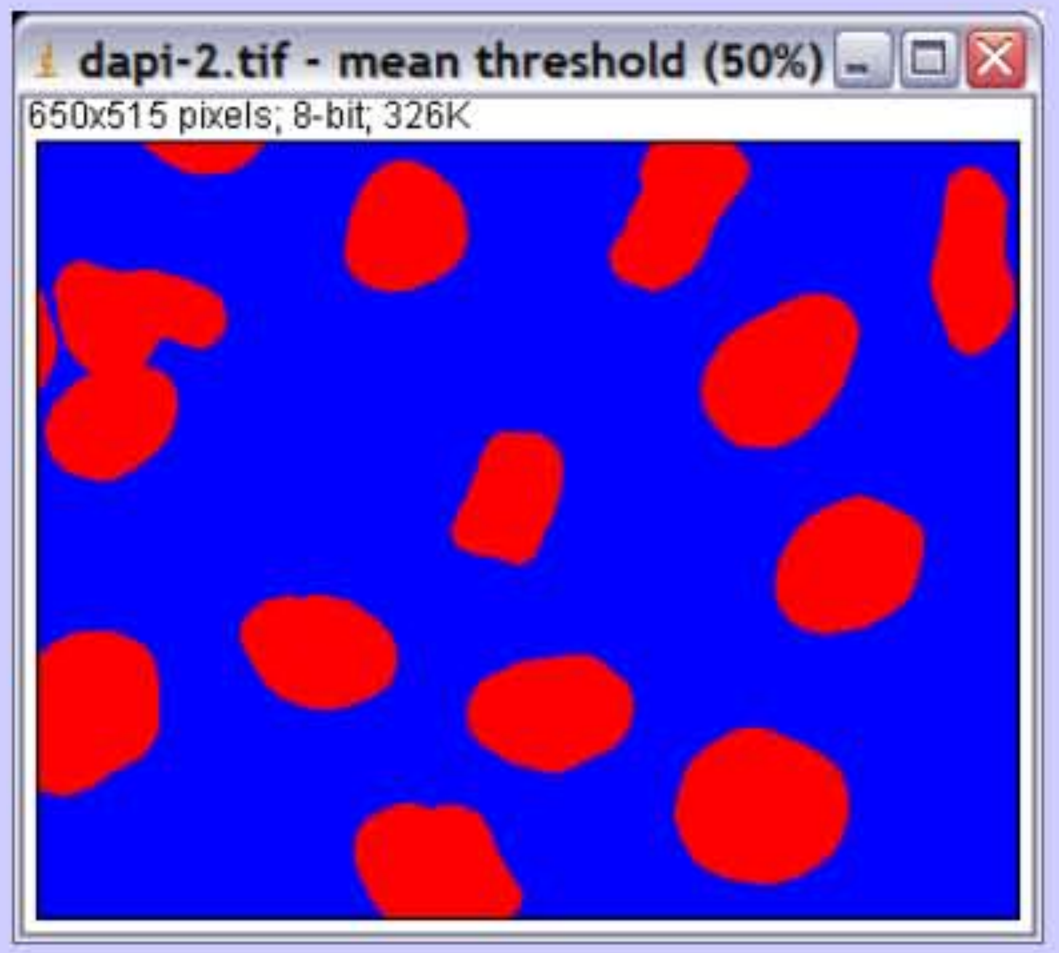
- threshold with mean intensity value



compare intensities nuclei outside ...

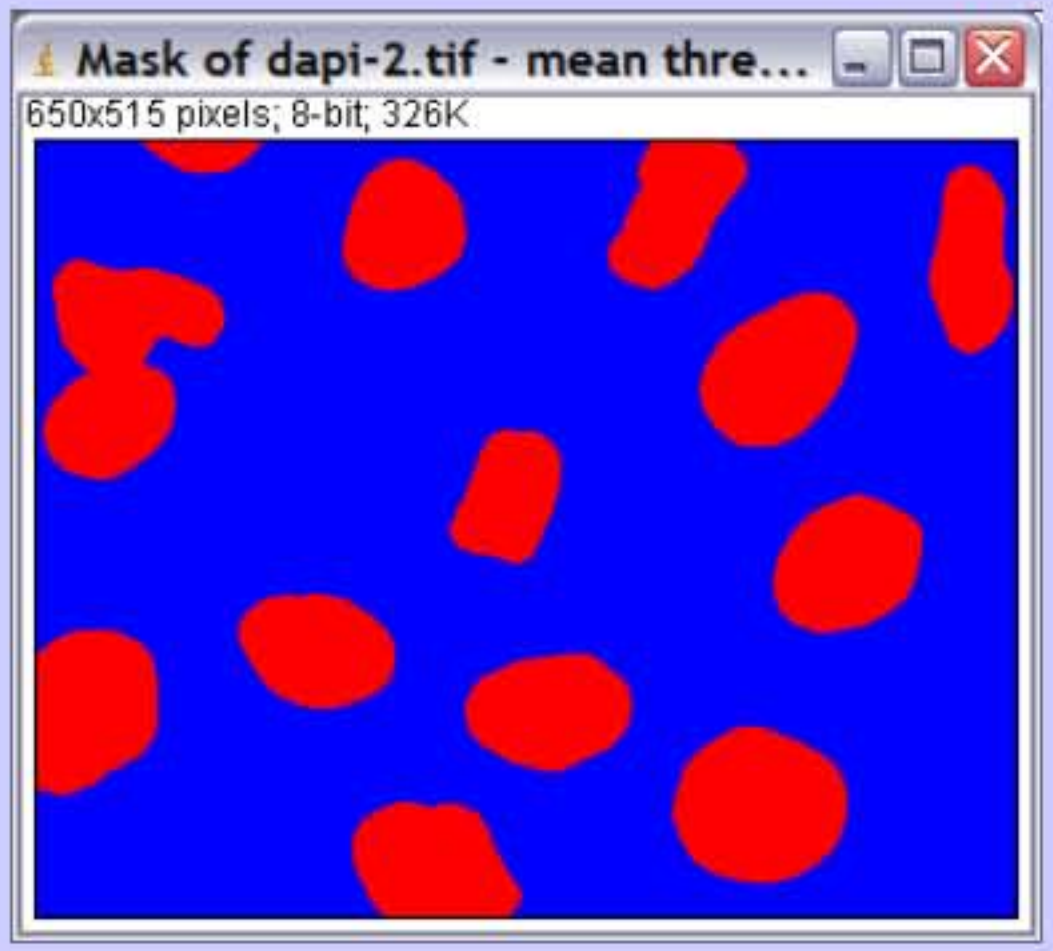
```
?  foreach image in list do        0  P
?  open image                      0  P
?  open image                      0  P
?  skip image not loaded           0  P
?  skip saturated                  0  P
?  convert image type              0  P
?  convert image type              0  P
?  find and subtract background    0  P
?  mean threshold                  0  P
?  invert image                    0  P
?  find objects                    0  P
?  select white pixel              0  P
?  transfer selection              0  P
```

Measuring intensity ratios



dapi-2.tif - mean threshold (50%)

650x515 pixels; 8-bit; 326K

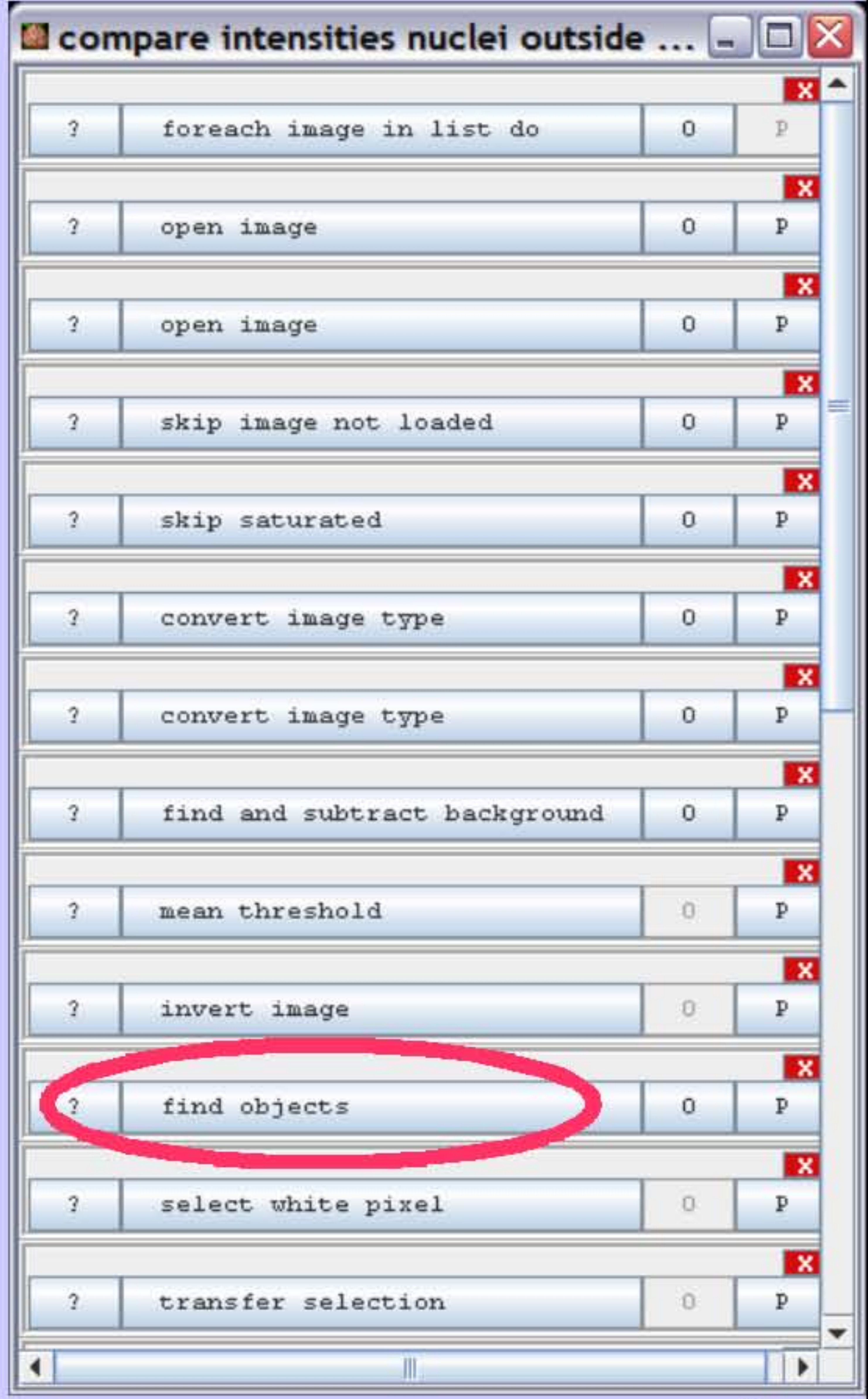


Mask of dapi-2.tif - mean thre...

650x515 pixels; 8-bit; 326K

find objects (analyze particles)
- exclude objects smaller min size

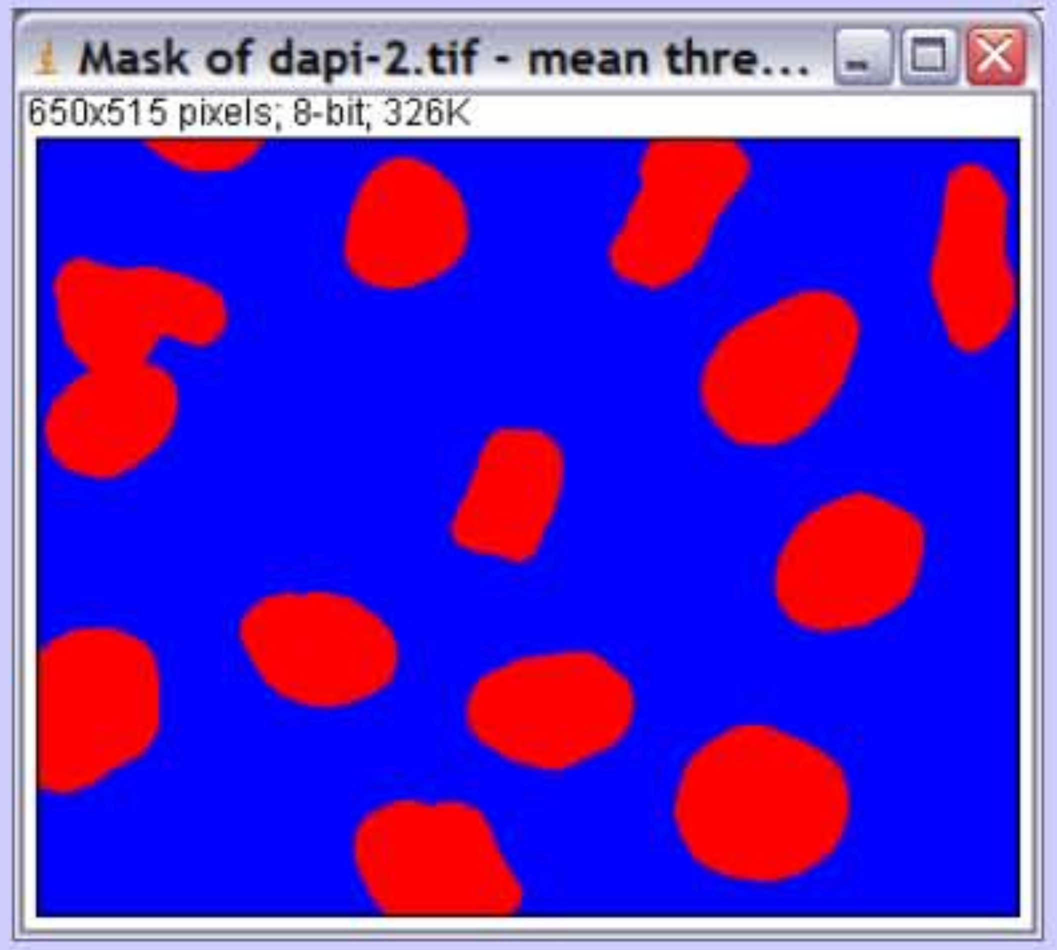
gets rid of objects that are not nuclei

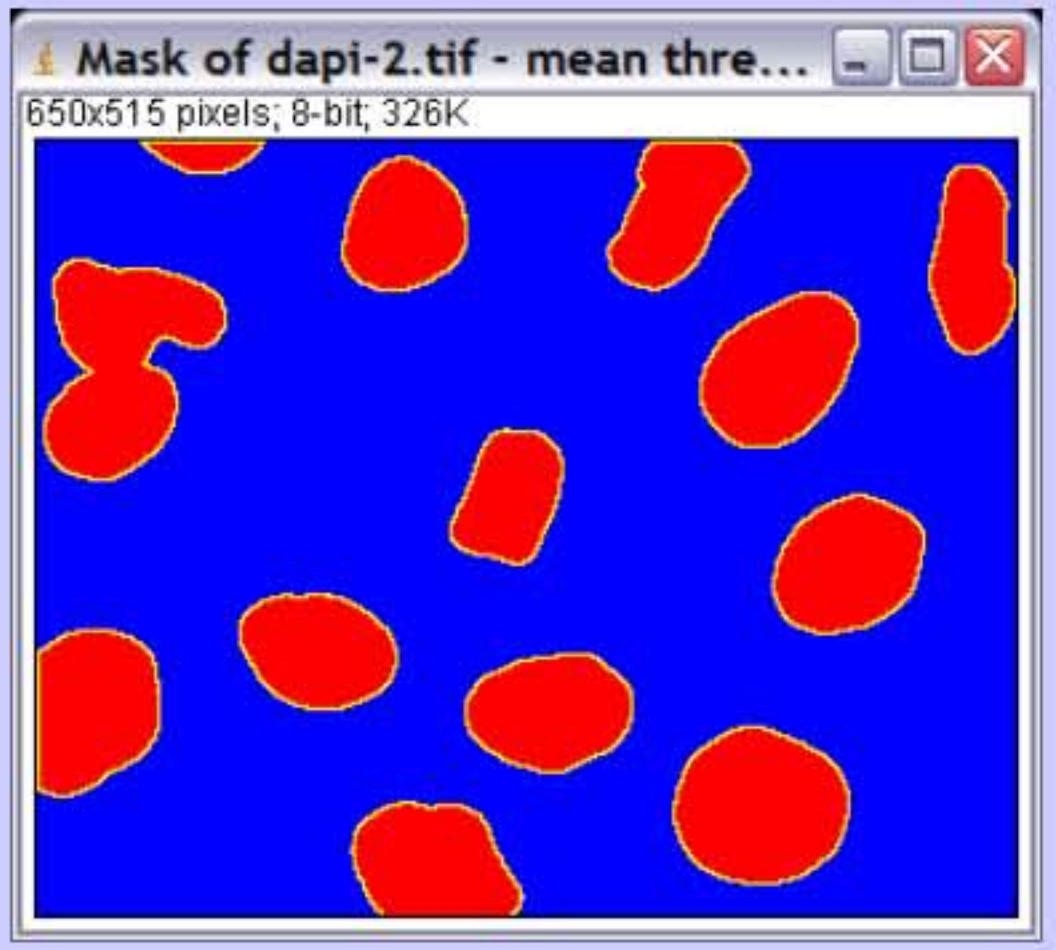


compare intensities nuclei outside ...

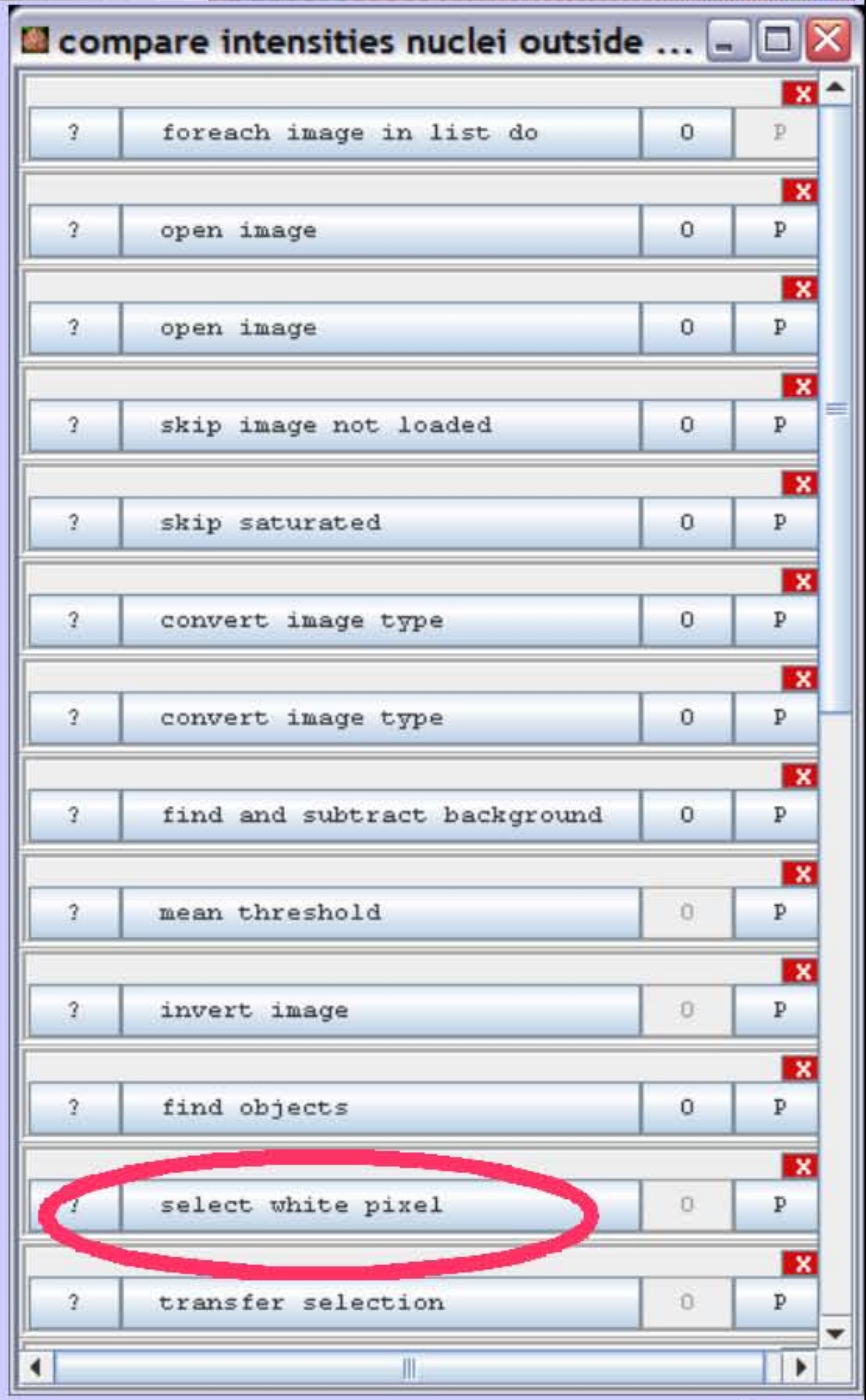
?	foreach image in list do	0	P
?	open image	0	P
?	open image	0	P
?	skip image not loaded	0	P
?	skip saturated	0	P
?	convert image type	0	P
?	convert image type	0	P
?	find and subtract background	0	P
?	mean threshold	0	P
?	invert image	0	P
?	find objects	0	P
?	select white pixel	0	P
?	transfer selection	0	P

Measuring intensity ratios


Mask of dapi-2.tif - mean thre...
650x515 pixels; 8-bit; 326K

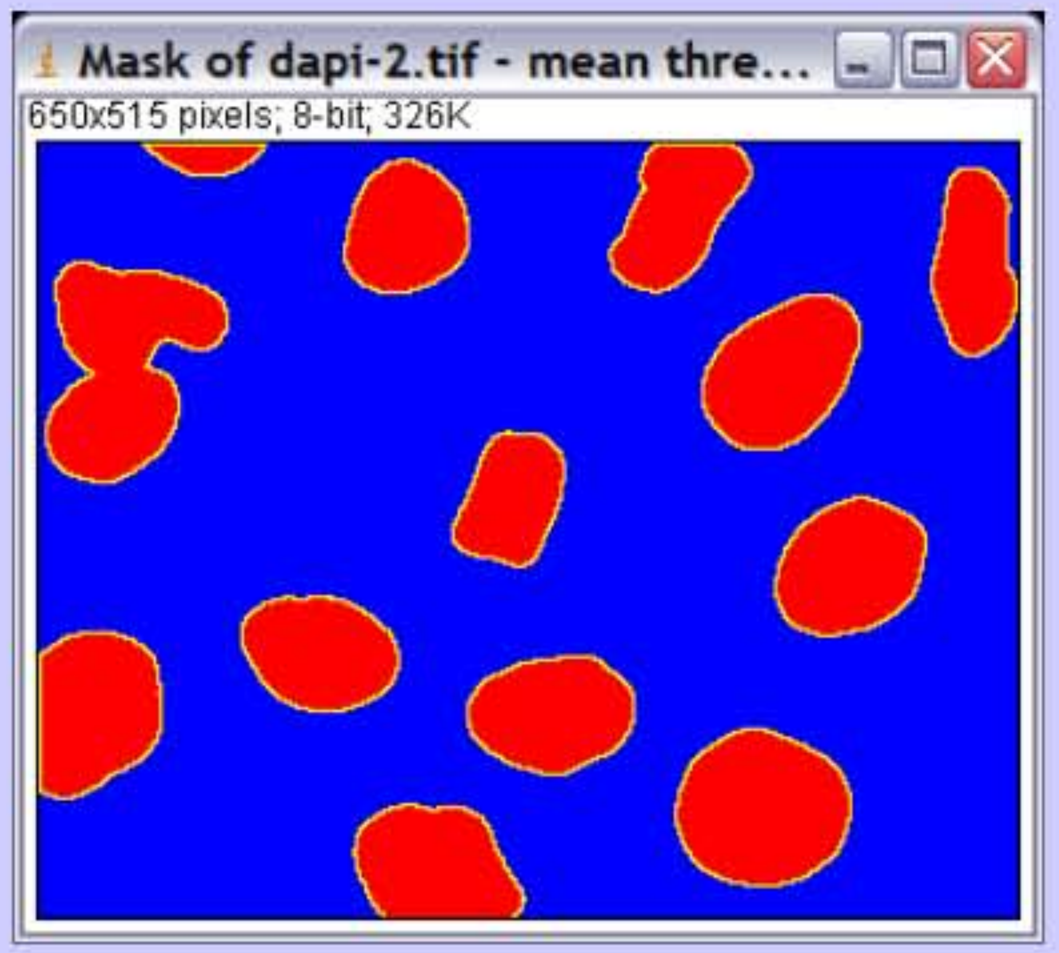

Mask of dapi-2.tif - mean thre...
650x515 pixels; 8-bit; 326K

create selection (roi) from mask


compare intensities nuclei outside ...

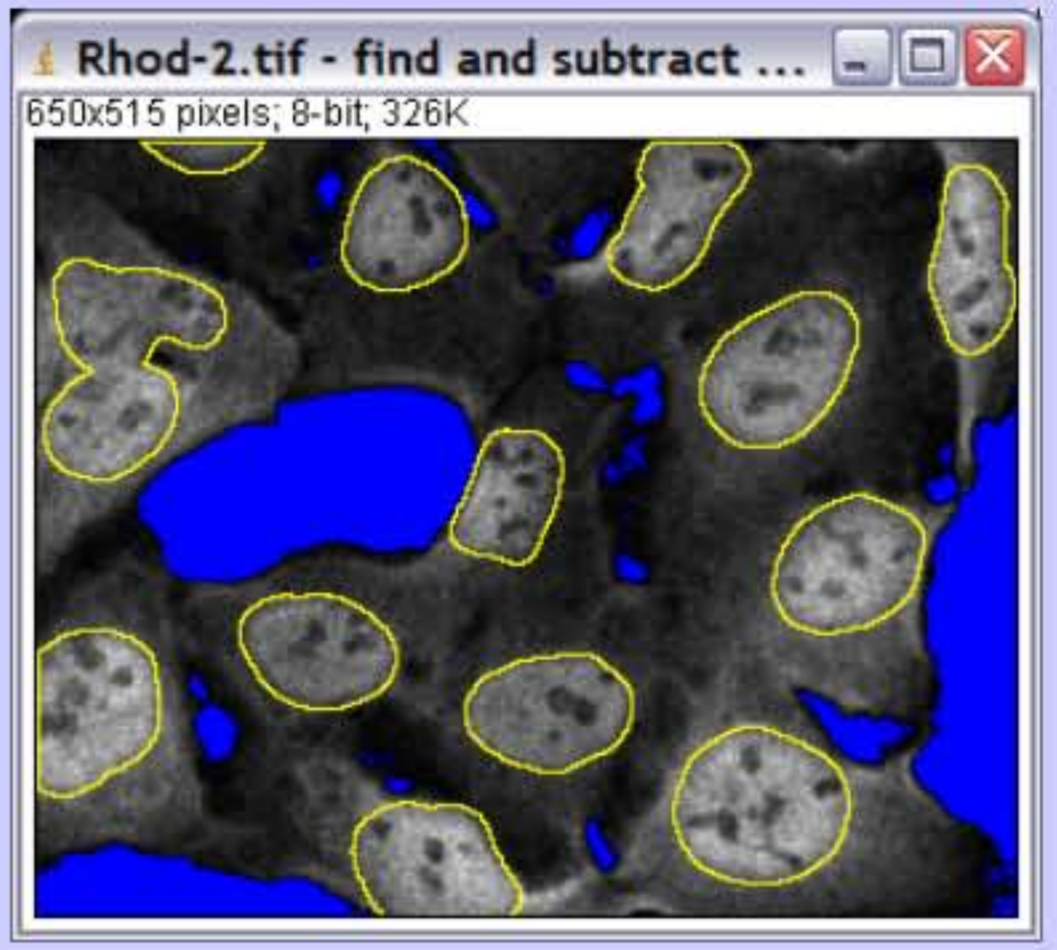
?	foreach image in list do	0	P
?	open image	0	P
?	open image	0	P
?	skip image not loaded	0	P
?	skip saturated	0	P
?	convert image type	0	P
?	convert image type	0	P
?	find and subtract background	0	P
?	mean threshold	0	P
?	invert image	0	P
?	find objects	0	P
?	select white pixel	0	P
?	transfer selection	0	P

Measuring intensity ratios



Mask of dapi-2.tif - mean thre...

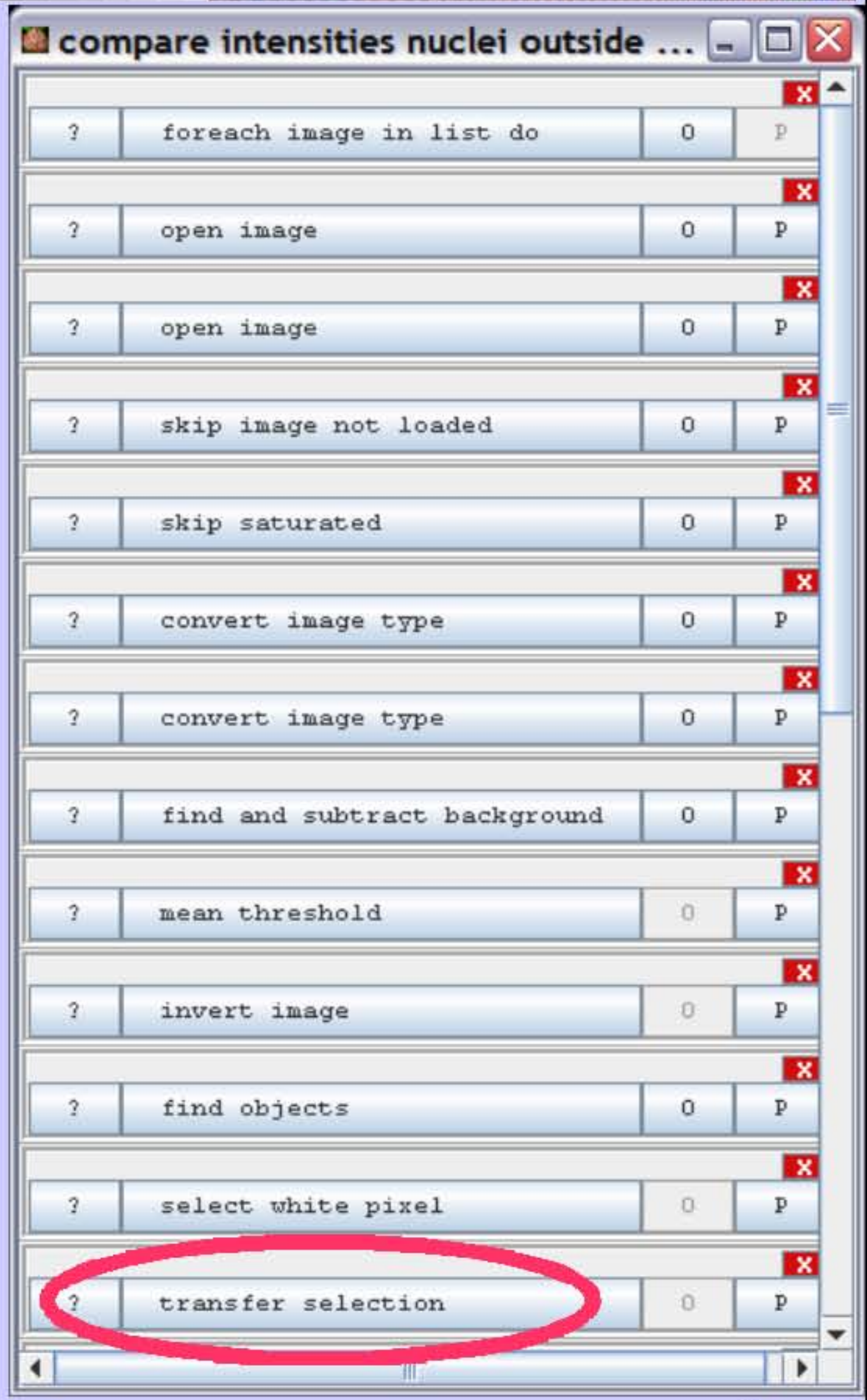
650x515 pixels; 8-bit; 326K



Rhod-2.tif - find and subtract ...

650x515 pixels; 8-bit; 326K

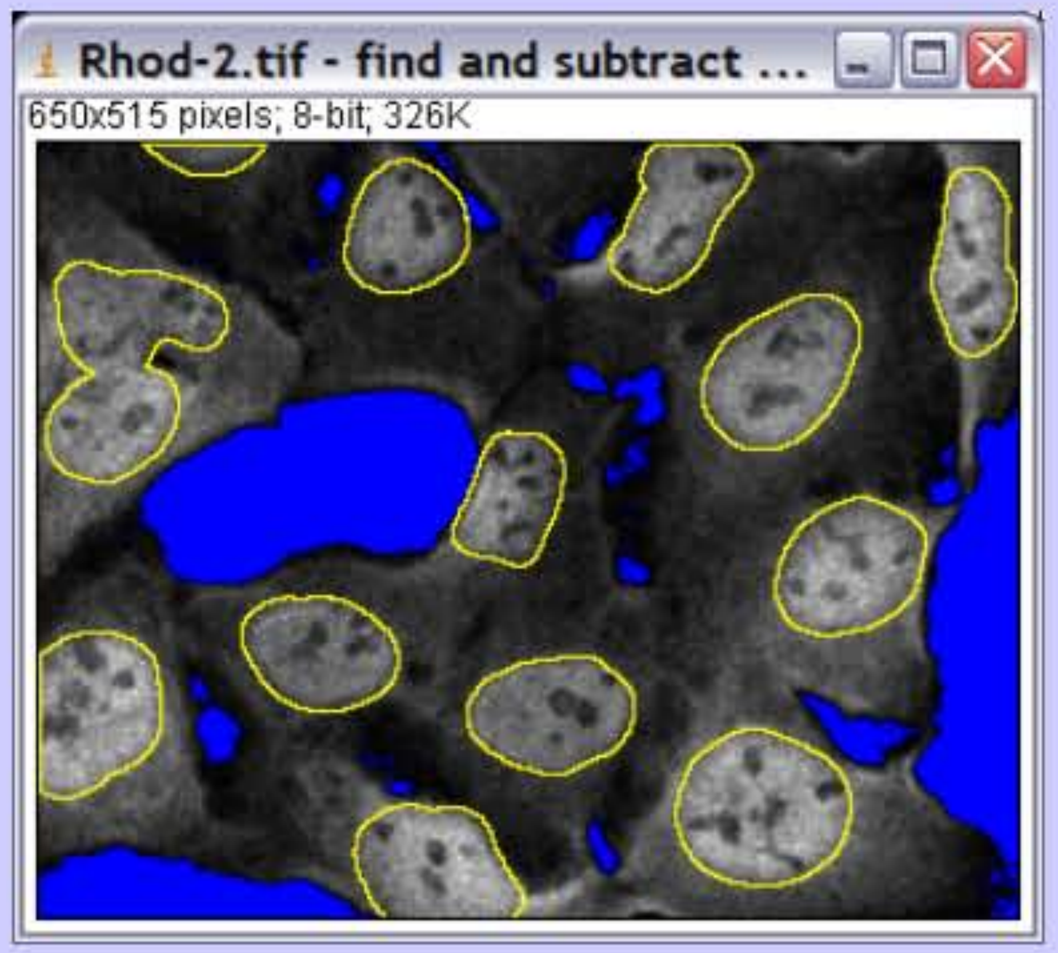
transfer selection to background corrected input image

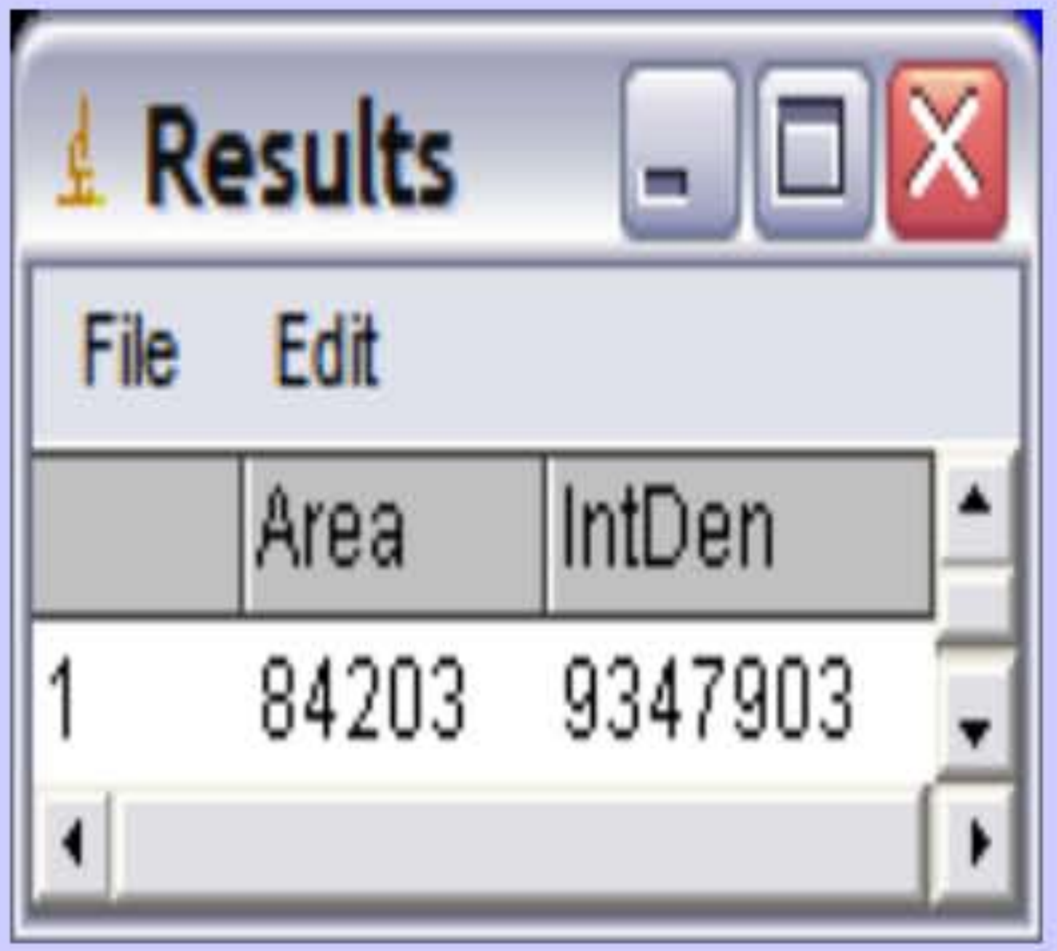


compare intensities nuclei outside ...

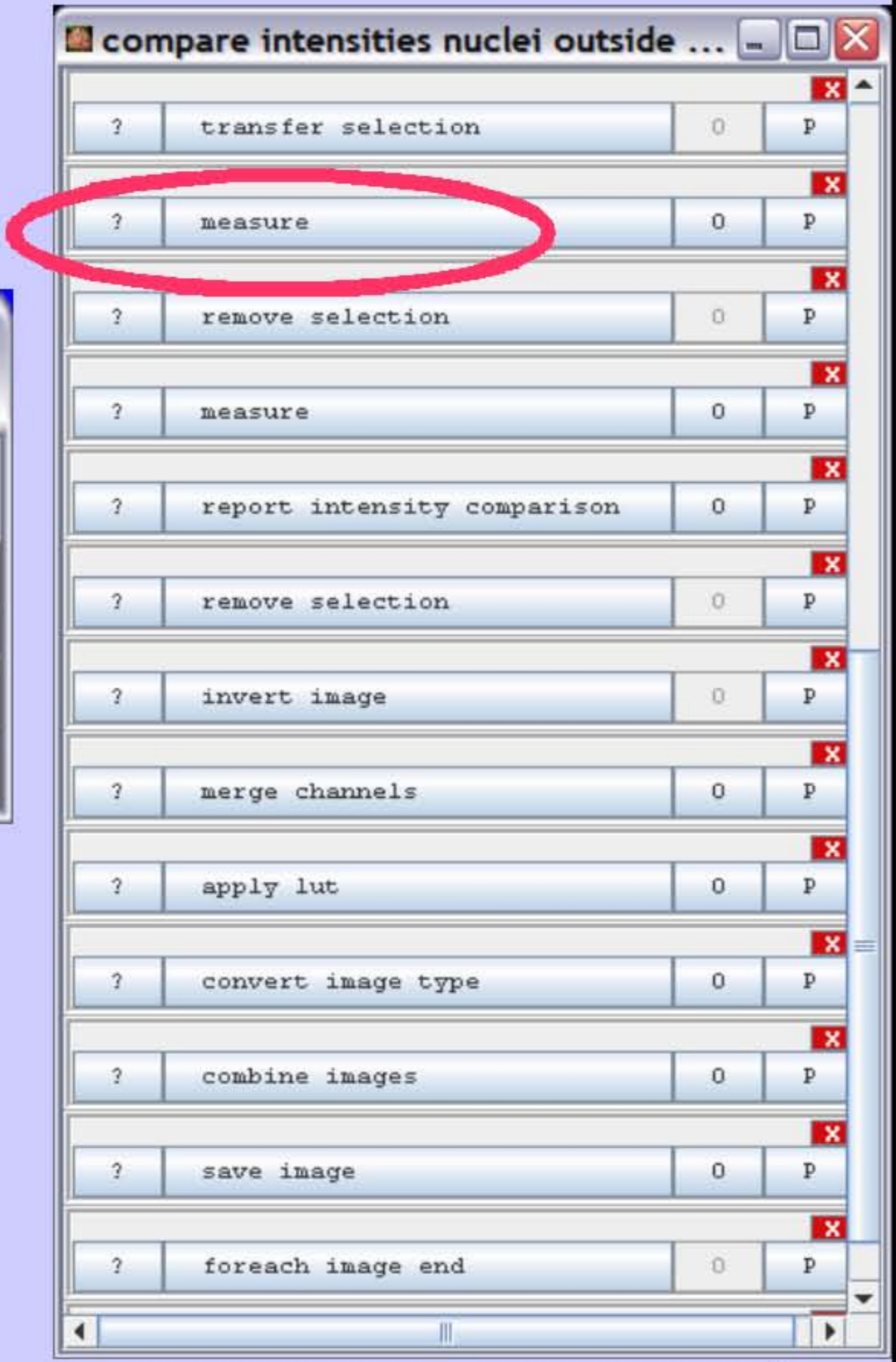
?	foreach image in list do	0	P
?	open image	0	P
?	open image	0	P
?	skip image not loaded	0	P
?	skip saturated	0	P
?	convert image type	0	P
?	convert image type	0	P
?	find and subtract background	0	P
?	mean threshold	0	P
?	invert image	0	P
?	find objects	0	P
?	select white pixel	0	P
?	transfer selection	0	P

Measuring intensity ratios


Rhod-2.tif - find and subtract ...
650x515 pixels; 8-bit; 326K



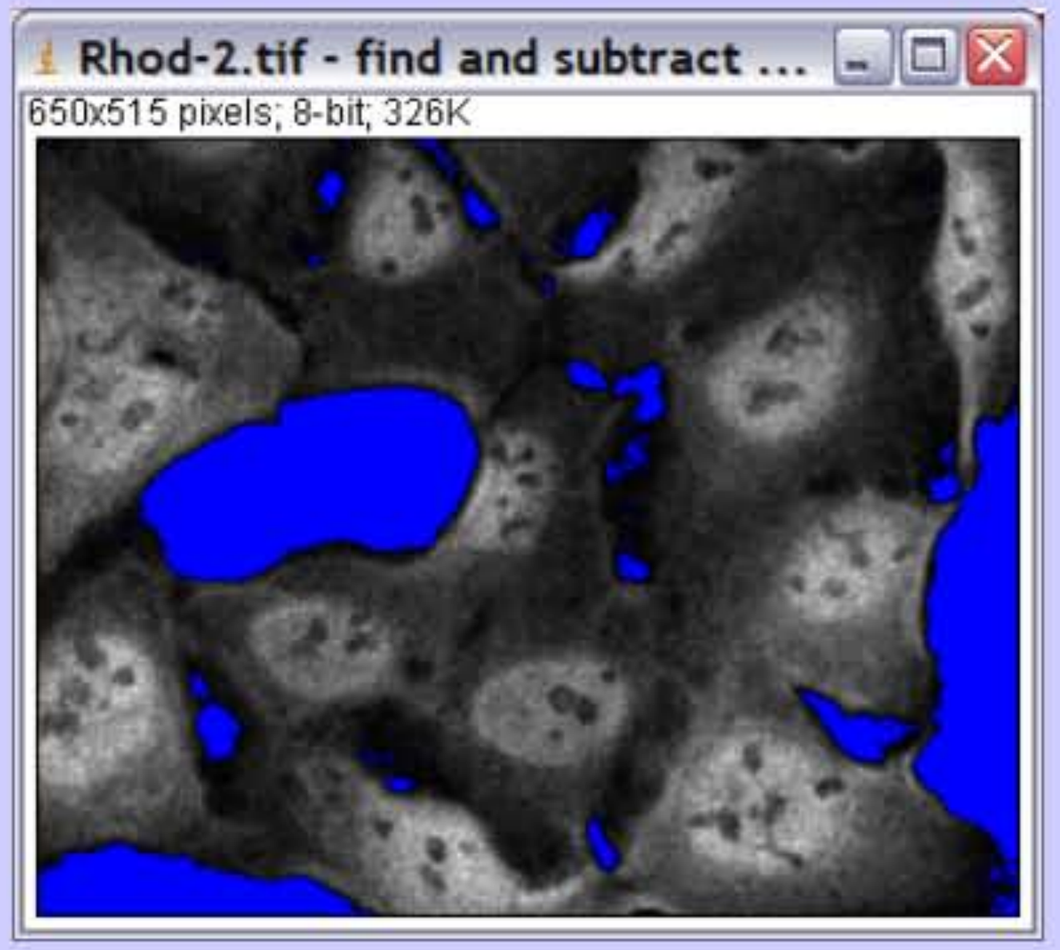
	Area	IntDen
1	84203	9347903



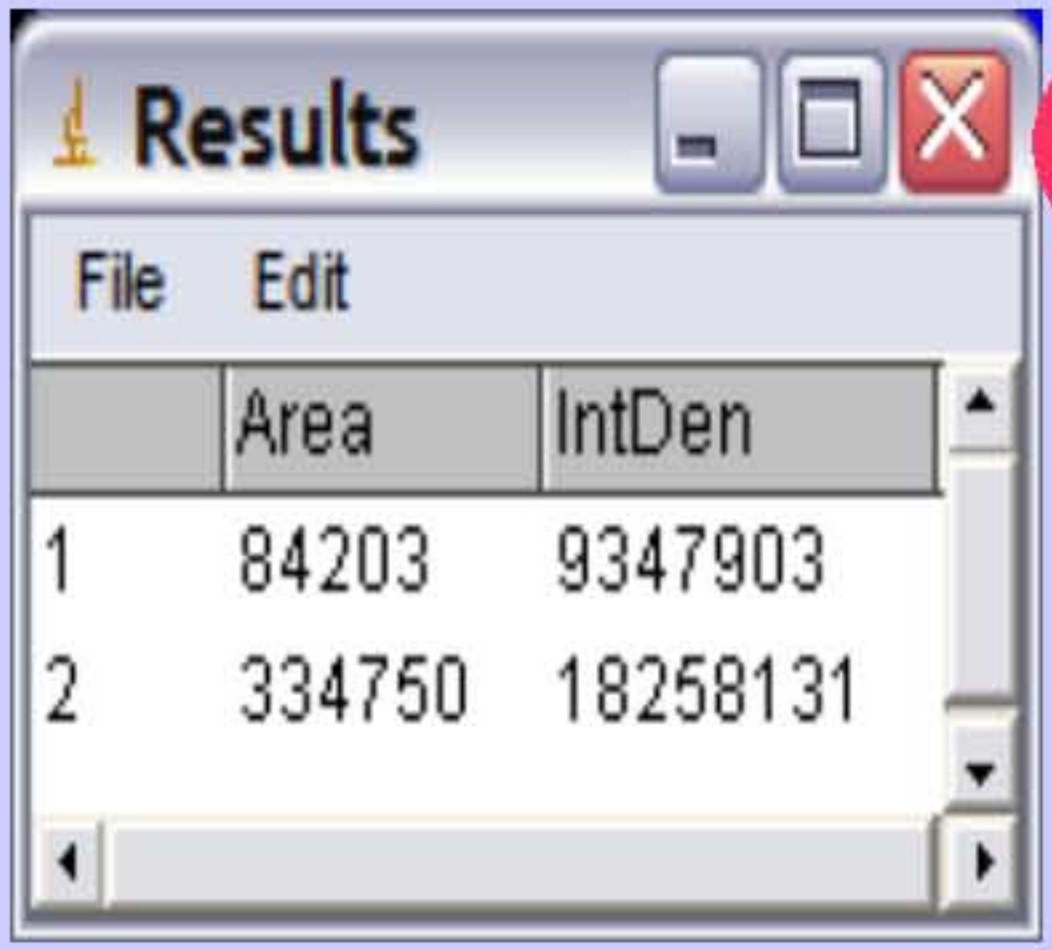
```
? transfer selection              0 P
? measure                         0 P
? remove selection                0 P
? measure                         0 P
? report intensity comparison     0 P
? remove selection                0 P
? invert image                    0 P
? merge channels                  0 P
? apply lut                       0 P
? convert image type              0 P
? combine images                  0 P
? save image                      0 P
? foreach image end               0 P
```

measure
- area
- total intensity of nuclei

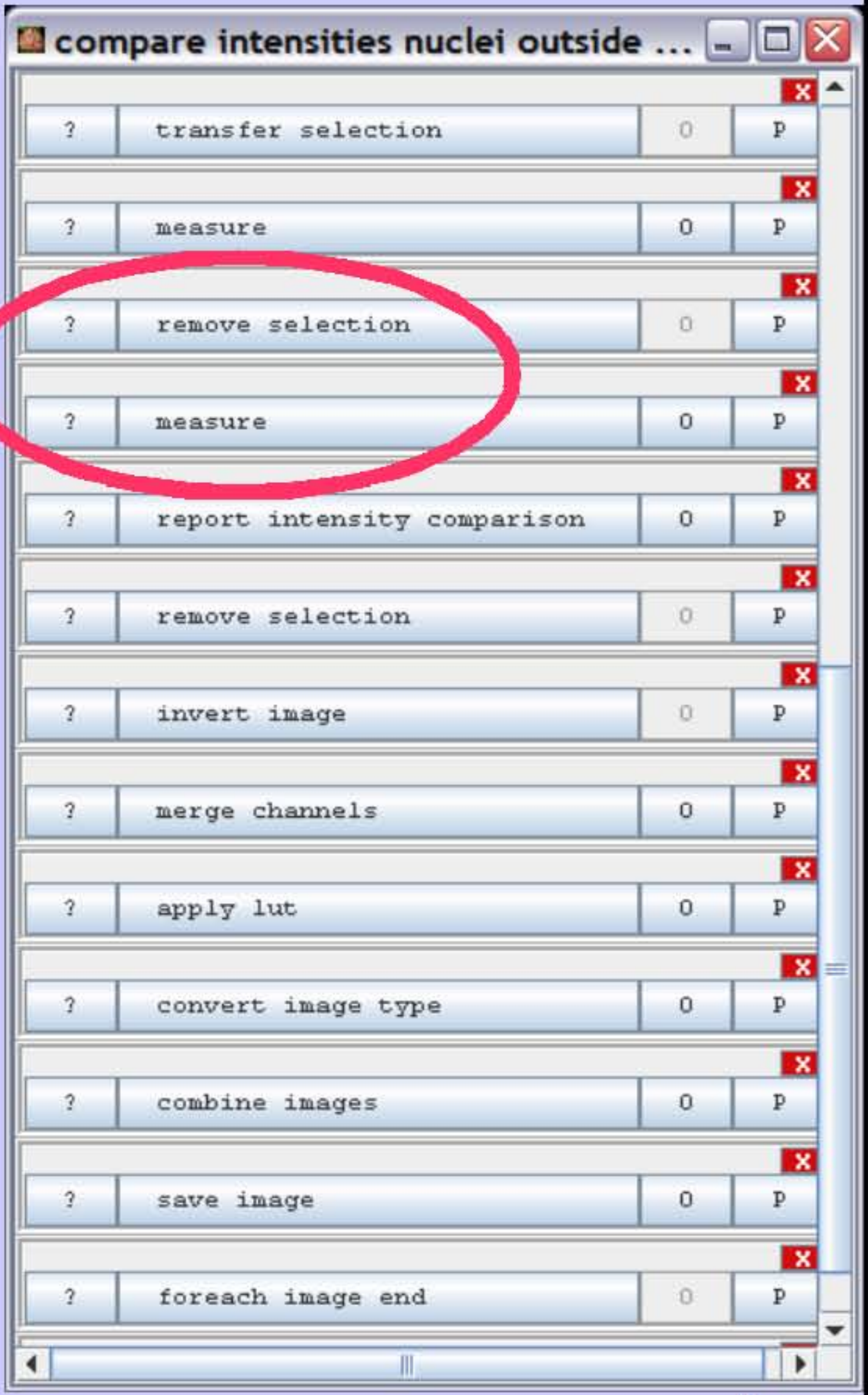
Measuring intensity ratios


Rhod-2.tif - find and subtract ...

650x515 pixels; 8-bit; 326K


Results

	Area	IntDen
1	84203	9347903
2	334750	18258131


compare intensities nuclei outside ...

?	transfer selection	0	P
?	measure	0	P
?	remove selection	0	P
?	measure	0	P
?	report intensity comparison	0	P
?	remove selection	0	P
?	invert image	0	P
?	merge channels	0	P
?	apply lut	0	P
?	convert image type	0	P
?	combine images	0	P
?	save image	0	P
?	foreach image end	0	P

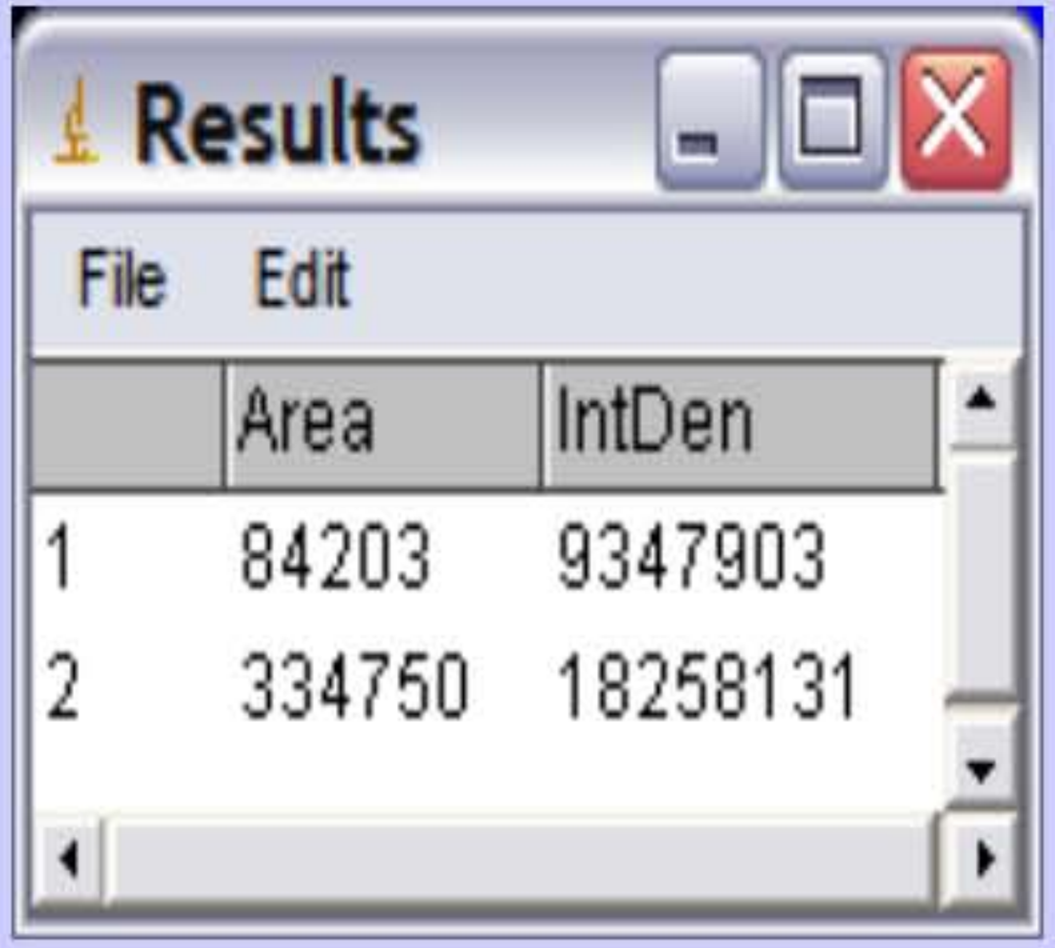
remove selection
measure area and intensity of the whole image

Measuring intensity ratios

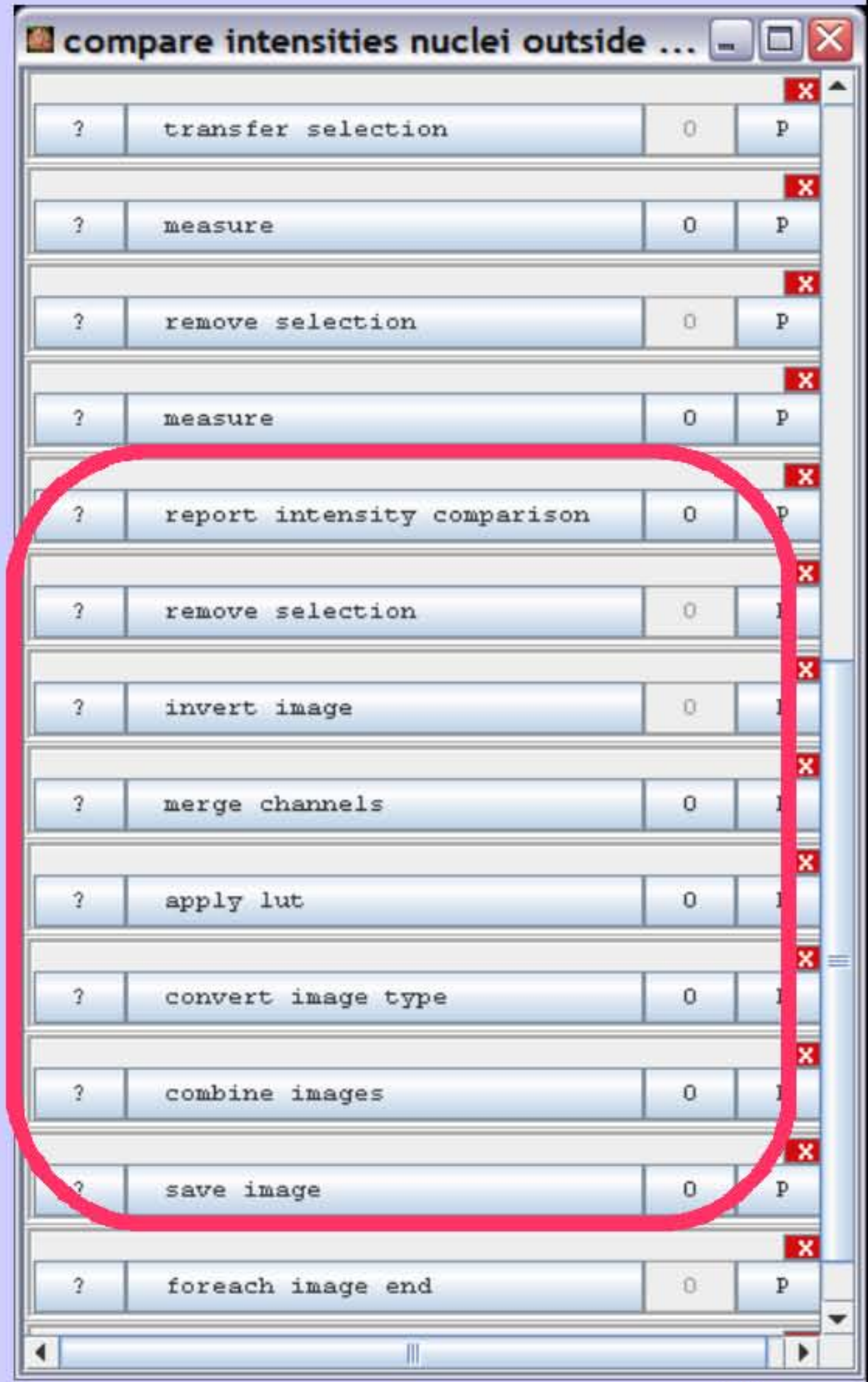
calculate percentage of intensity
- in nuclei
- outside nuclei

$p1 = d1 / d2$

$p2 = (d2 - d1) / d2$



	Area	IntDen
1	84203	9347903
2	334750	18258131



Measuring intensity ratios

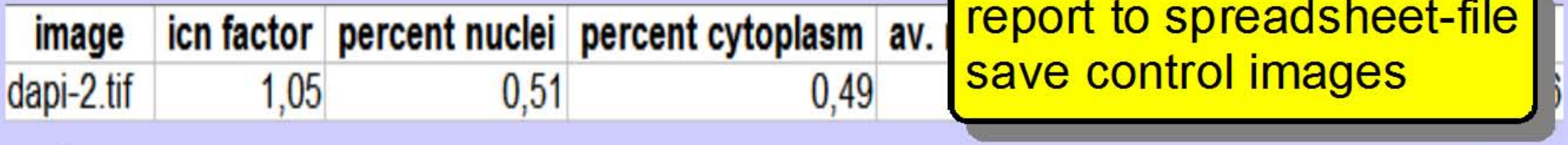
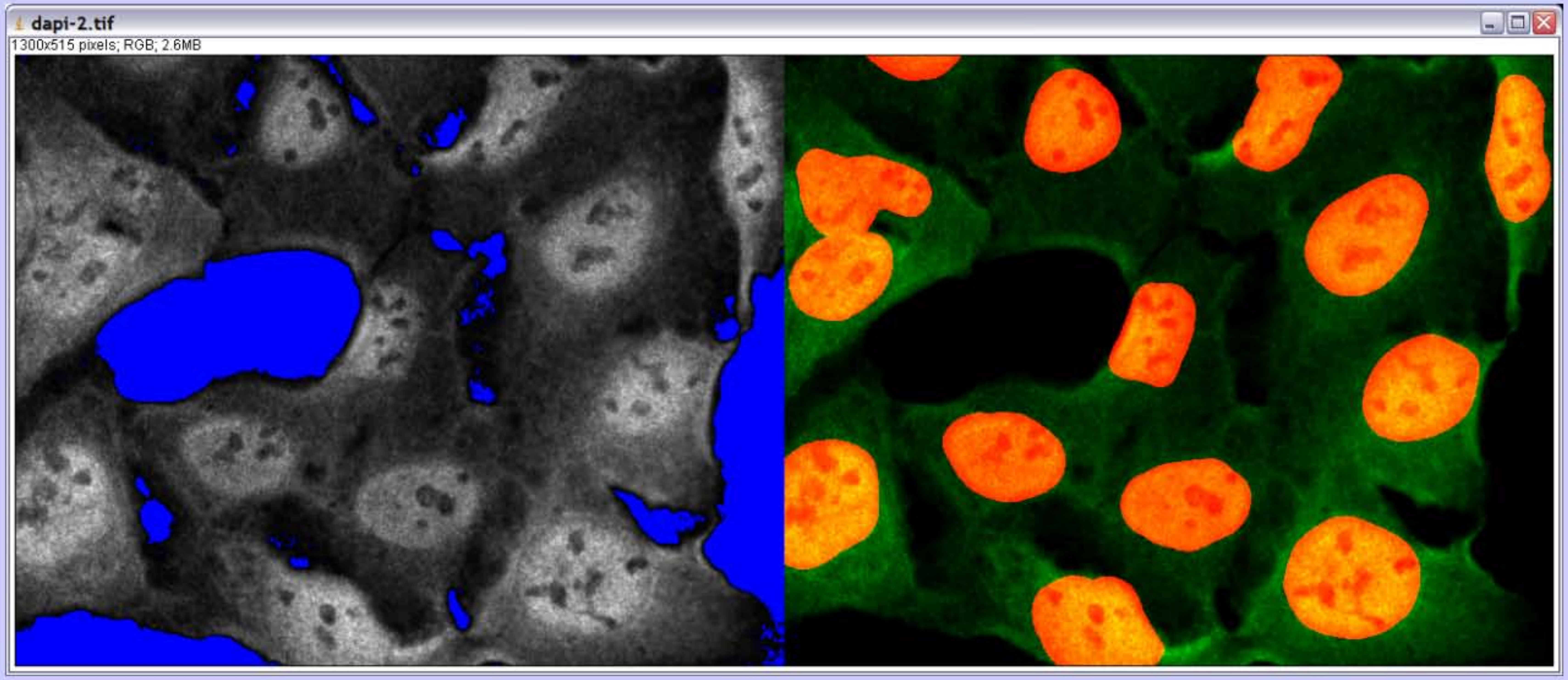


image	icn factor	percent nuclei	percent cytoplasm	av.
dapi-2.tif	1,05	0,51	0,49	6

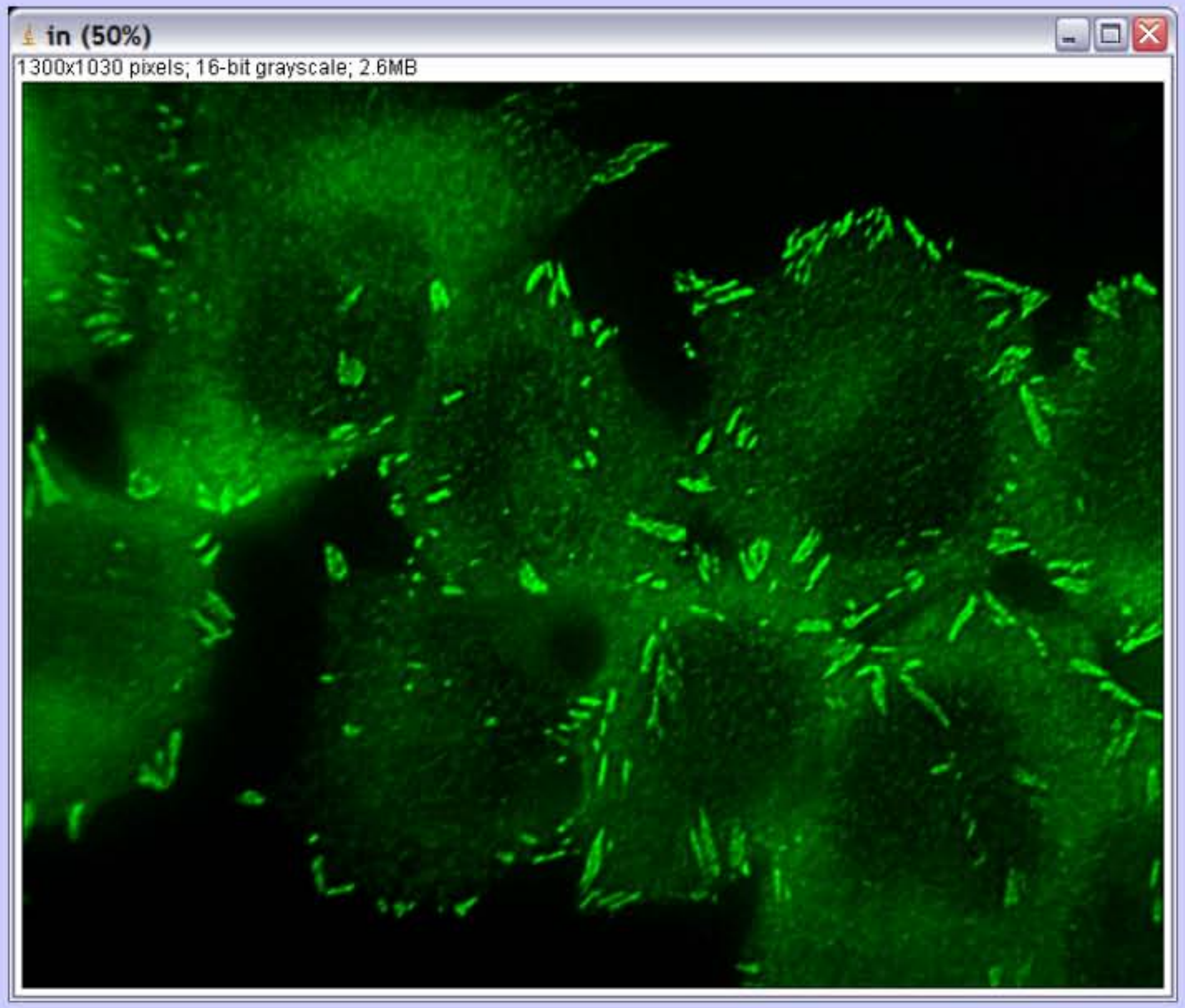
report to spreadsheet-file
save control images



dapi-2.tif

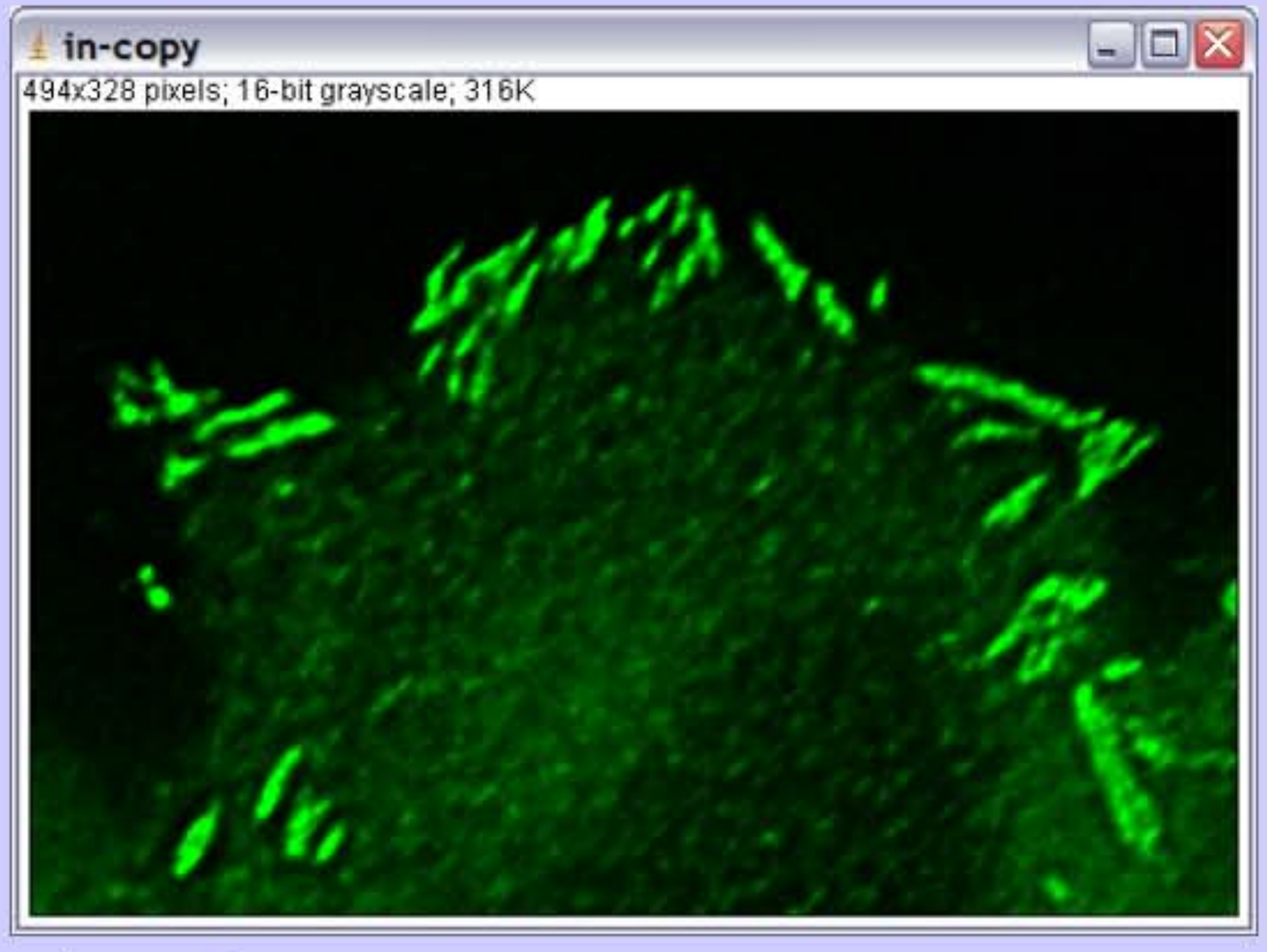
1300x515 pixels; RGB; 2.6MB

Measuring stained proteins



in (50%)

1300x1030 pixels; 16-bit grayscale; 2.6MB



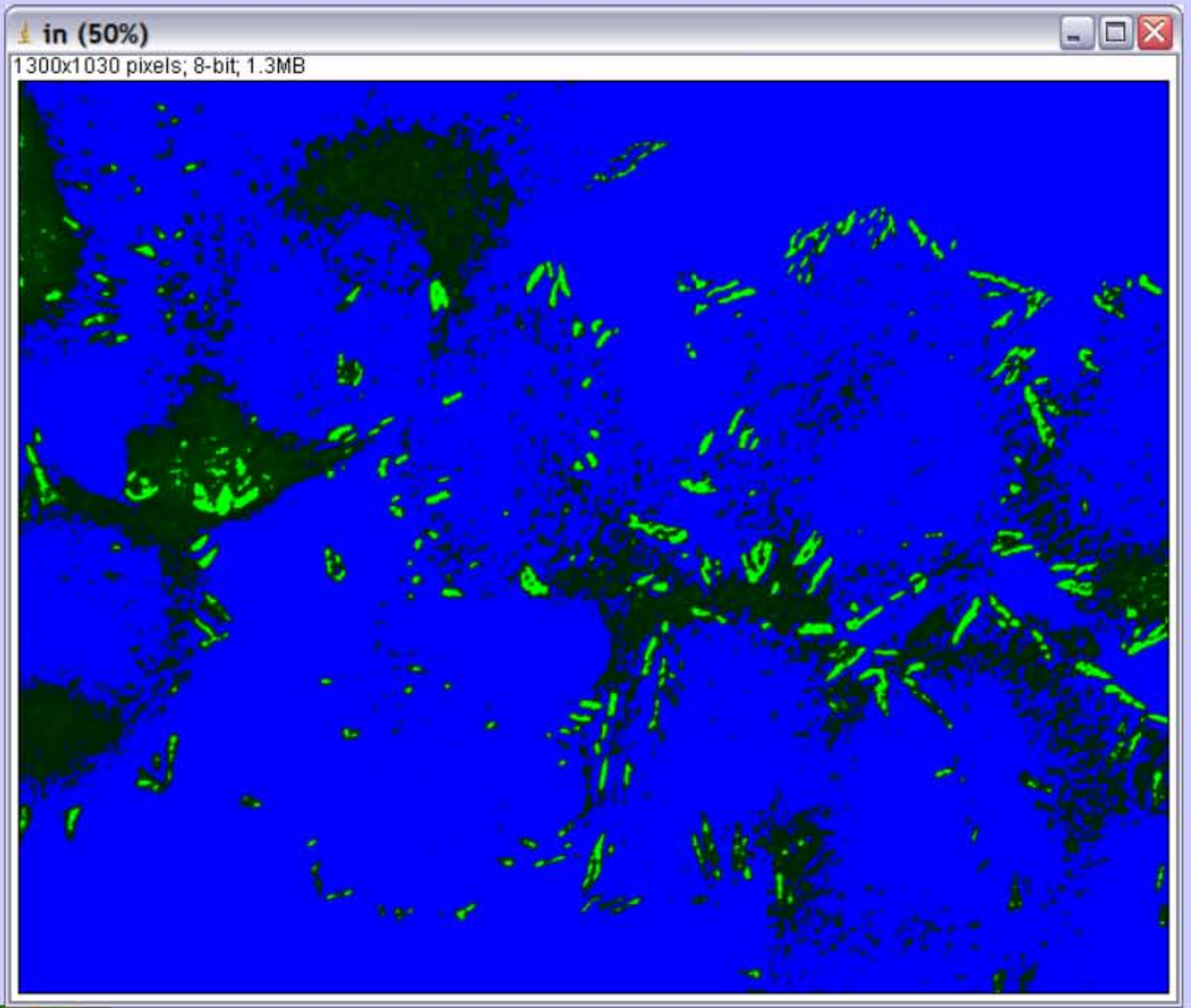
in-copy

494x328 pixels; 16-bit grayscale; 316K

task:

- count plaques
- measure size of plaques

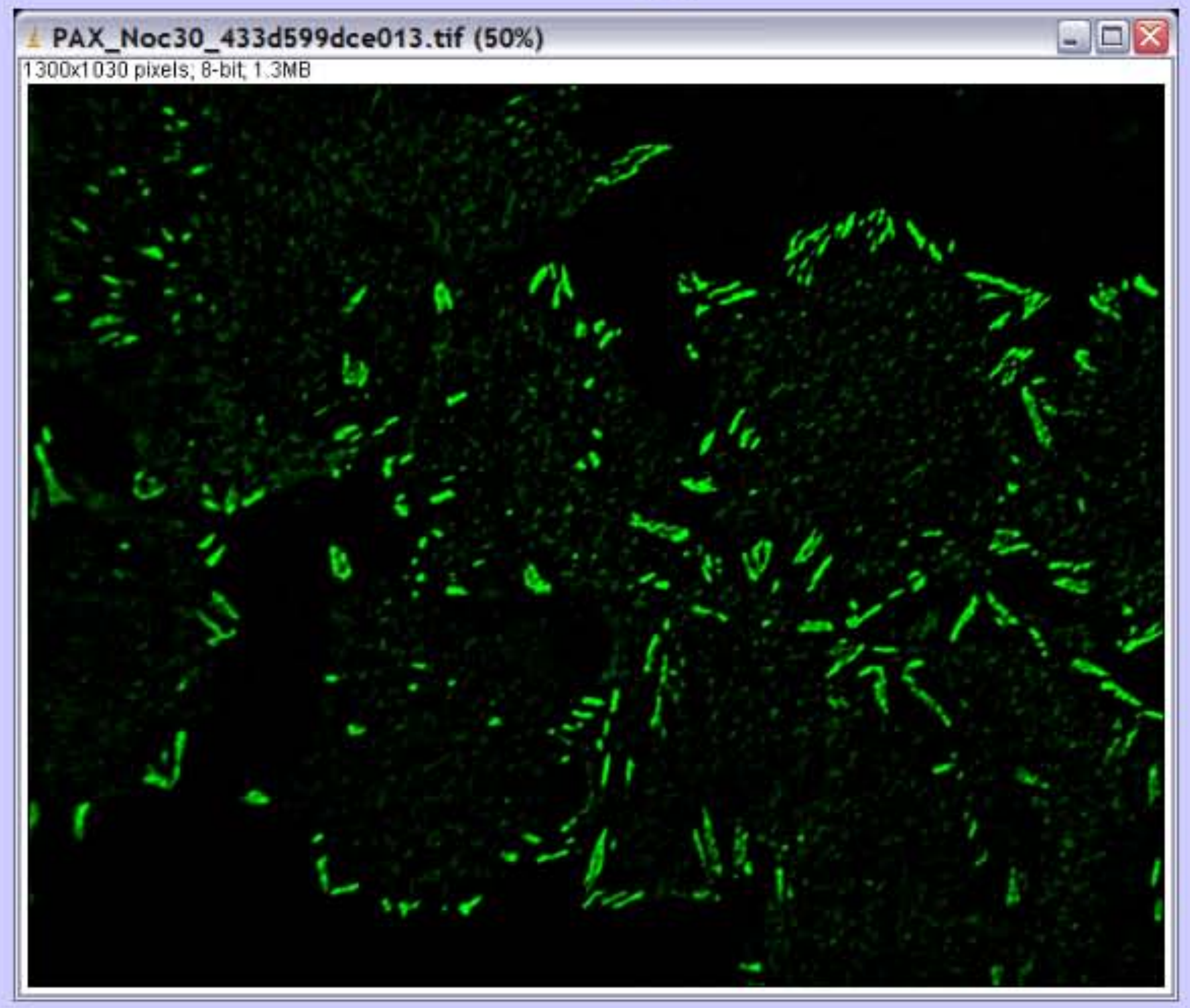
Measuring stained proteins

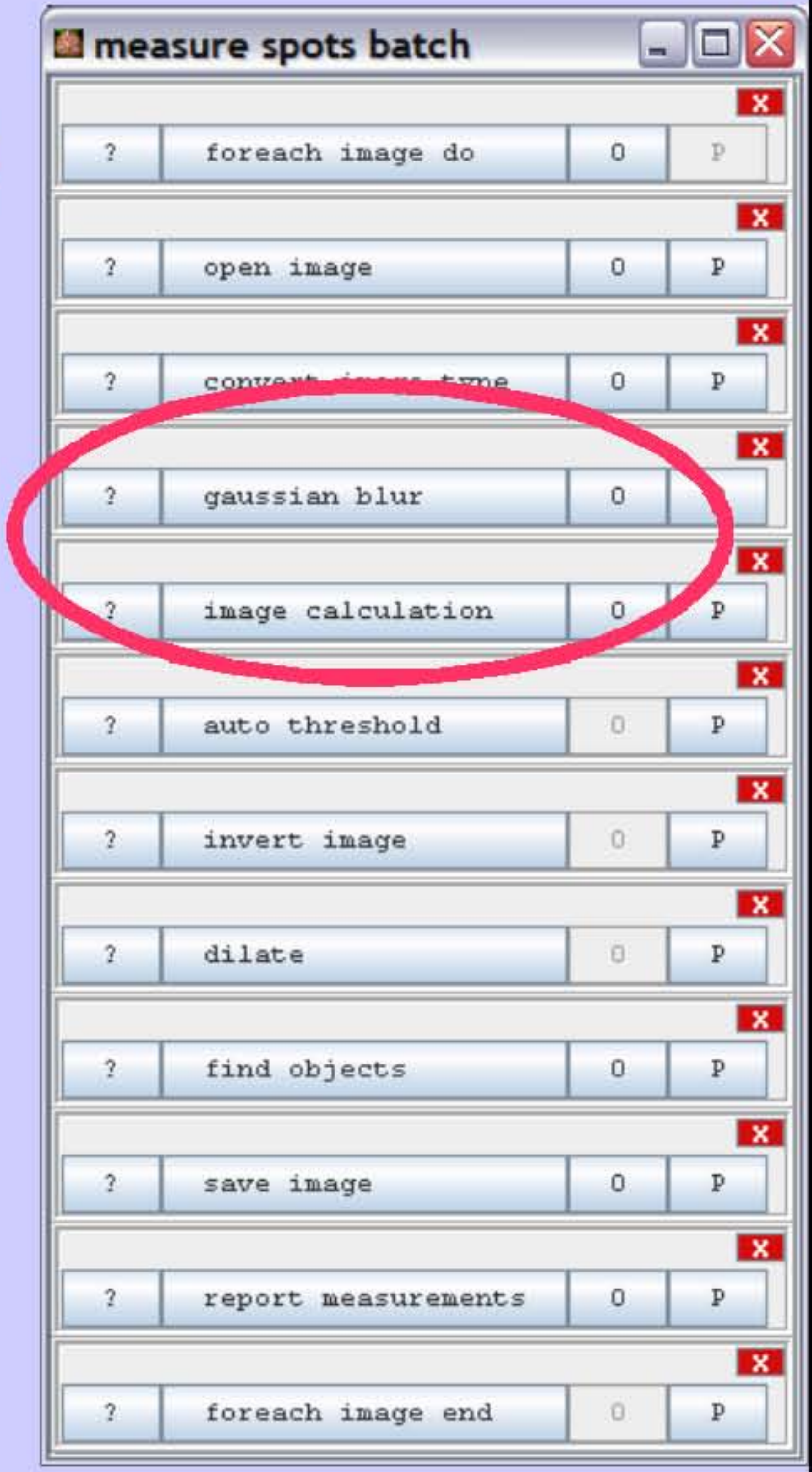


problem:

- same intensities in plaques and cytoplasm
- simple threshold doesn't separate plaques from background

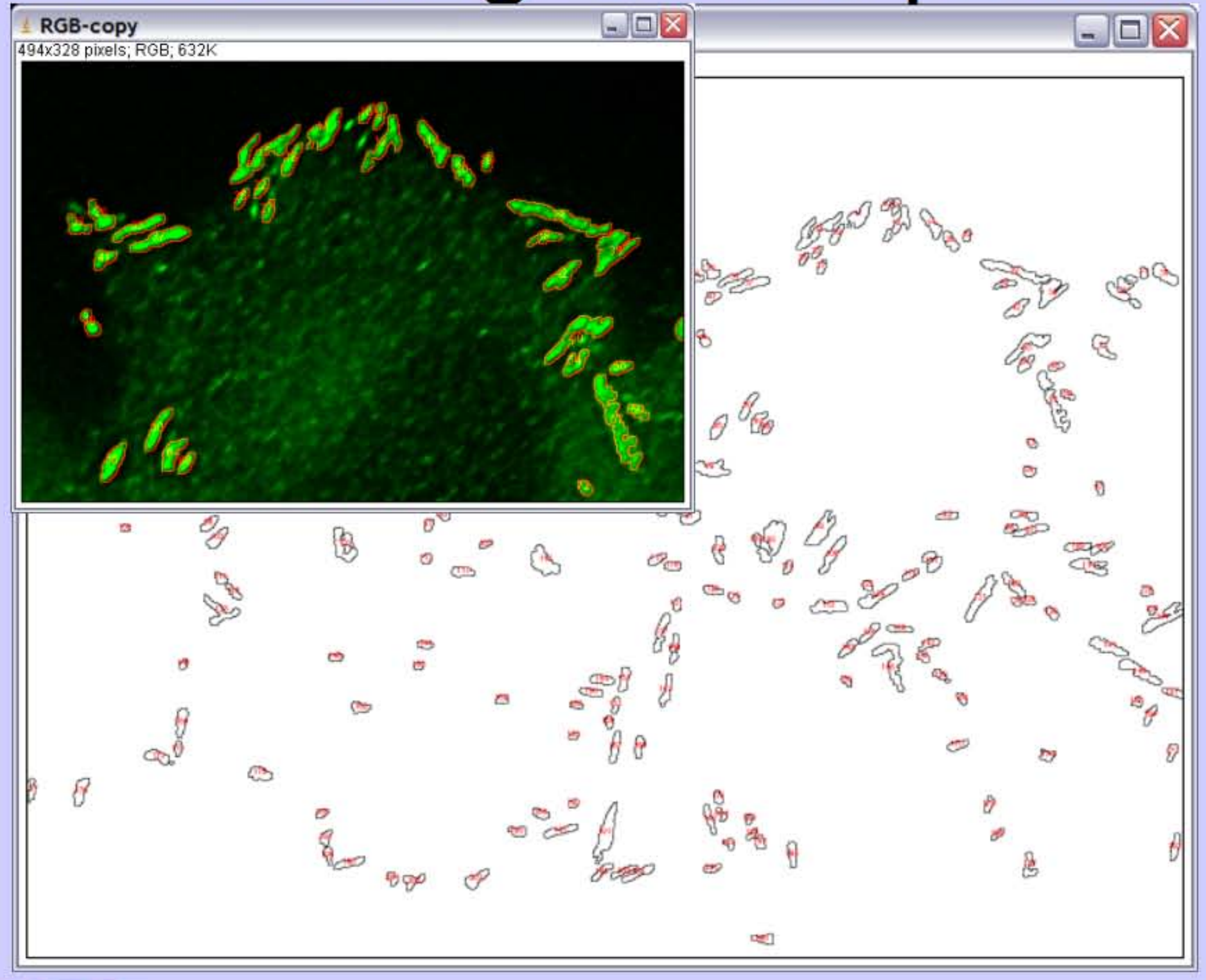
Measuring stained proteins

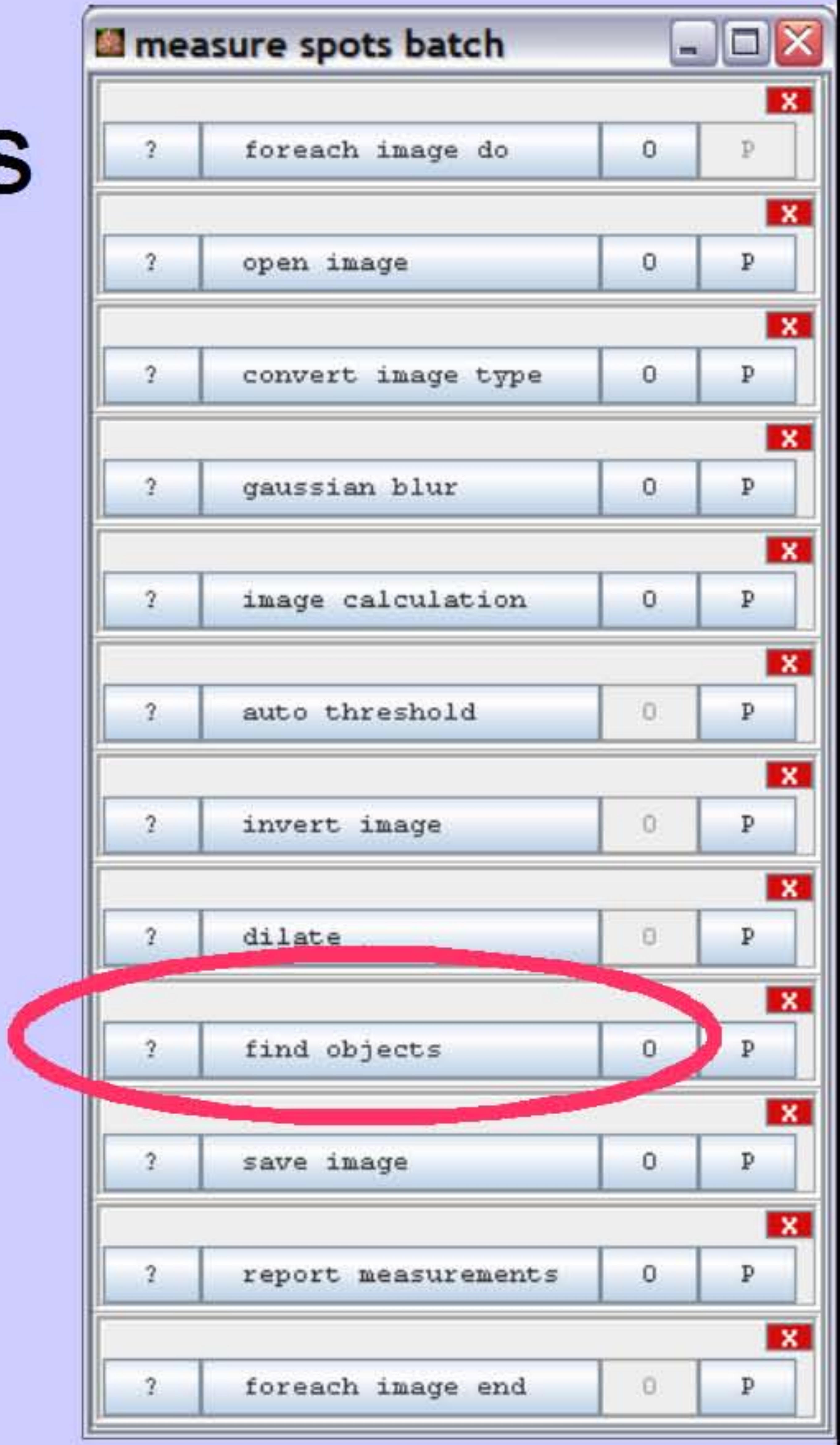

PAX_Noc30_433d599dce013.tif (50%)
1300x1030 pixels; 8-bit; 1.3MB



- subtract a blurred version of the image from the original
- only small, isolated dots of the background remain

Measuring stained proteins

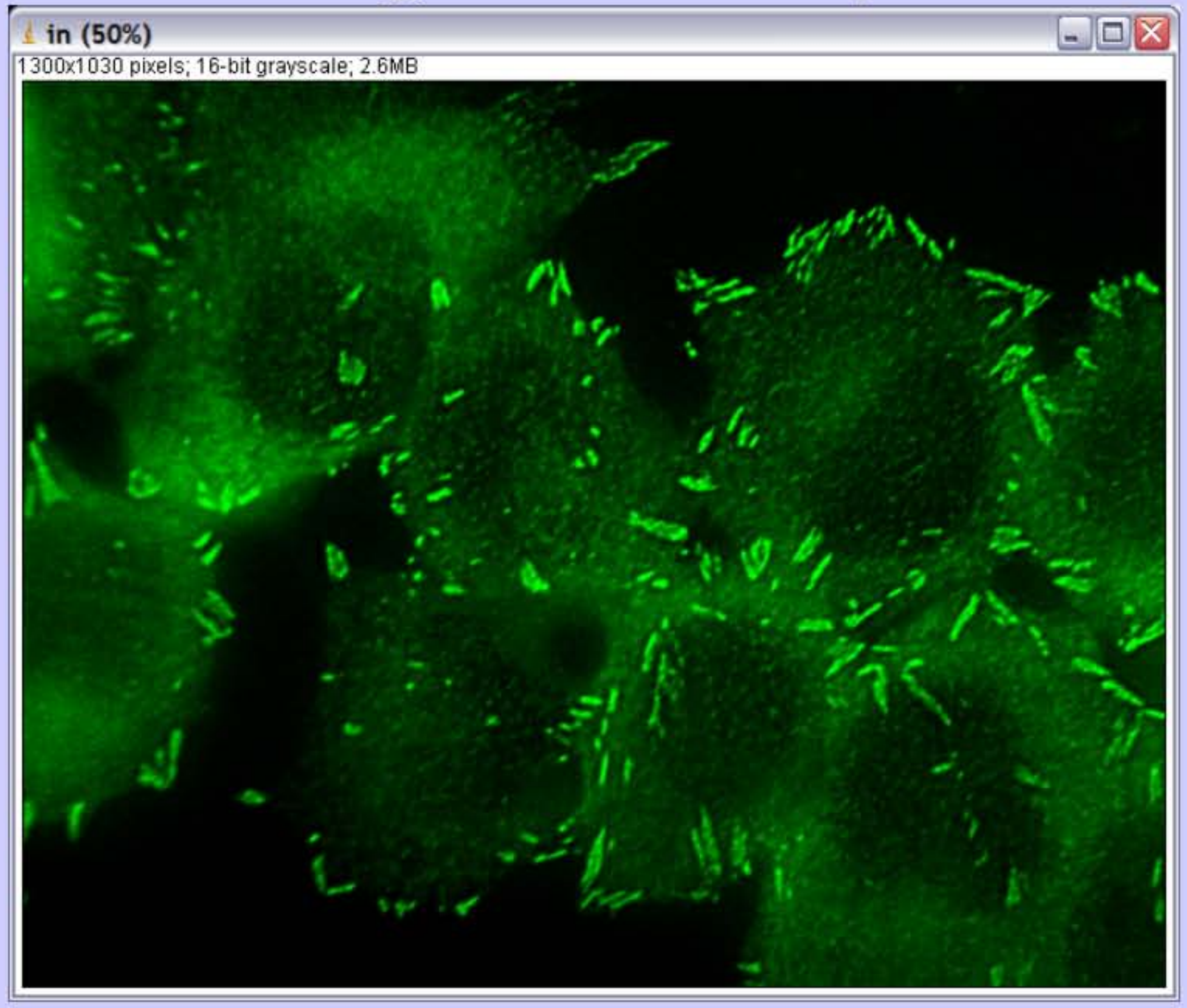




measure spots batch

?	foreach image do	0	P
?	open image	0	P
?	convert image type	0	P
?	gaussian blur	0	P
?	image calculation	0	P
?	auto threshold	0	P
?	invert image	0	P
?	dilate	0	P
?	find objects	0	P
?	save image	0	P
?	report measurements	0	P
?	foreach image end	0	P

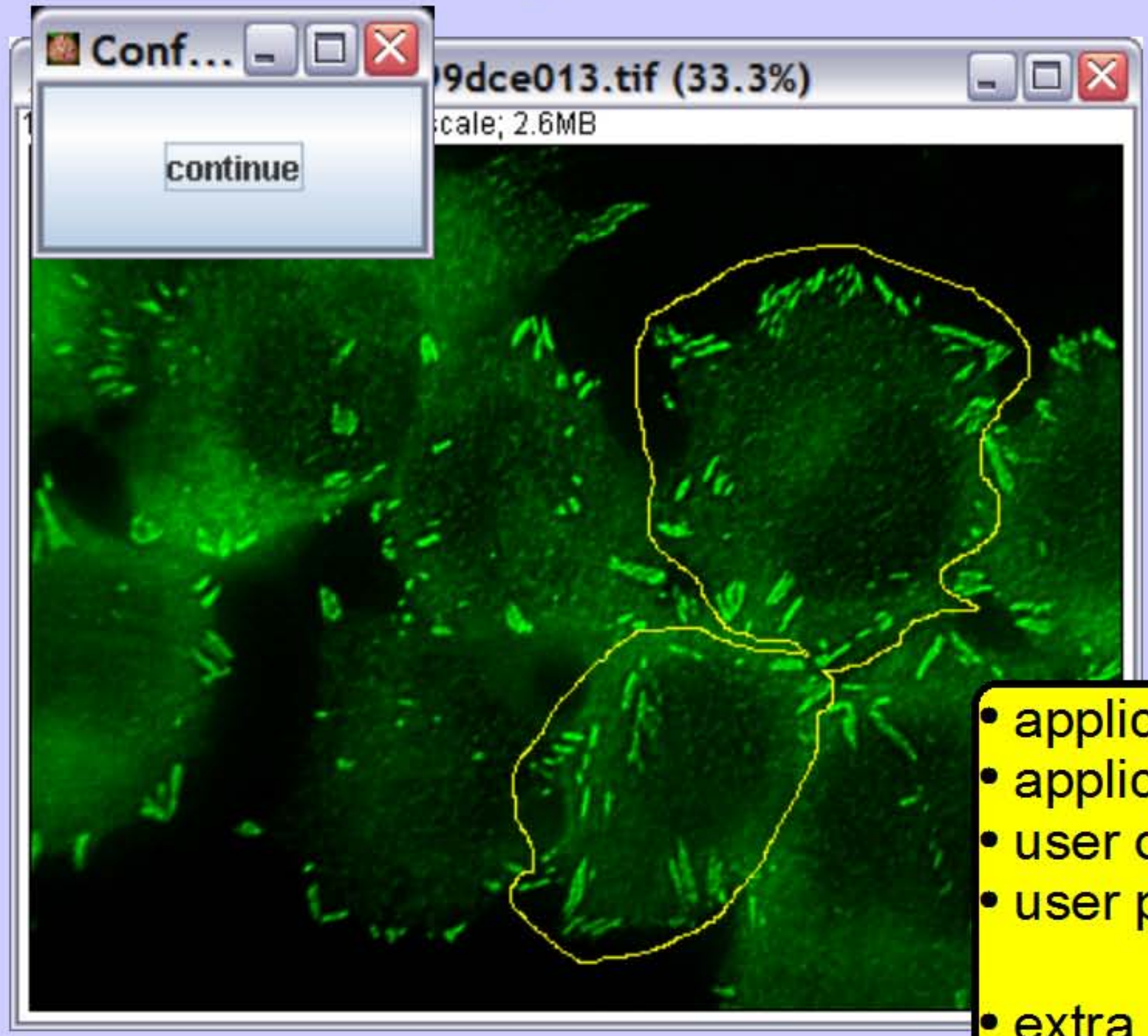
Measuring stained proteins

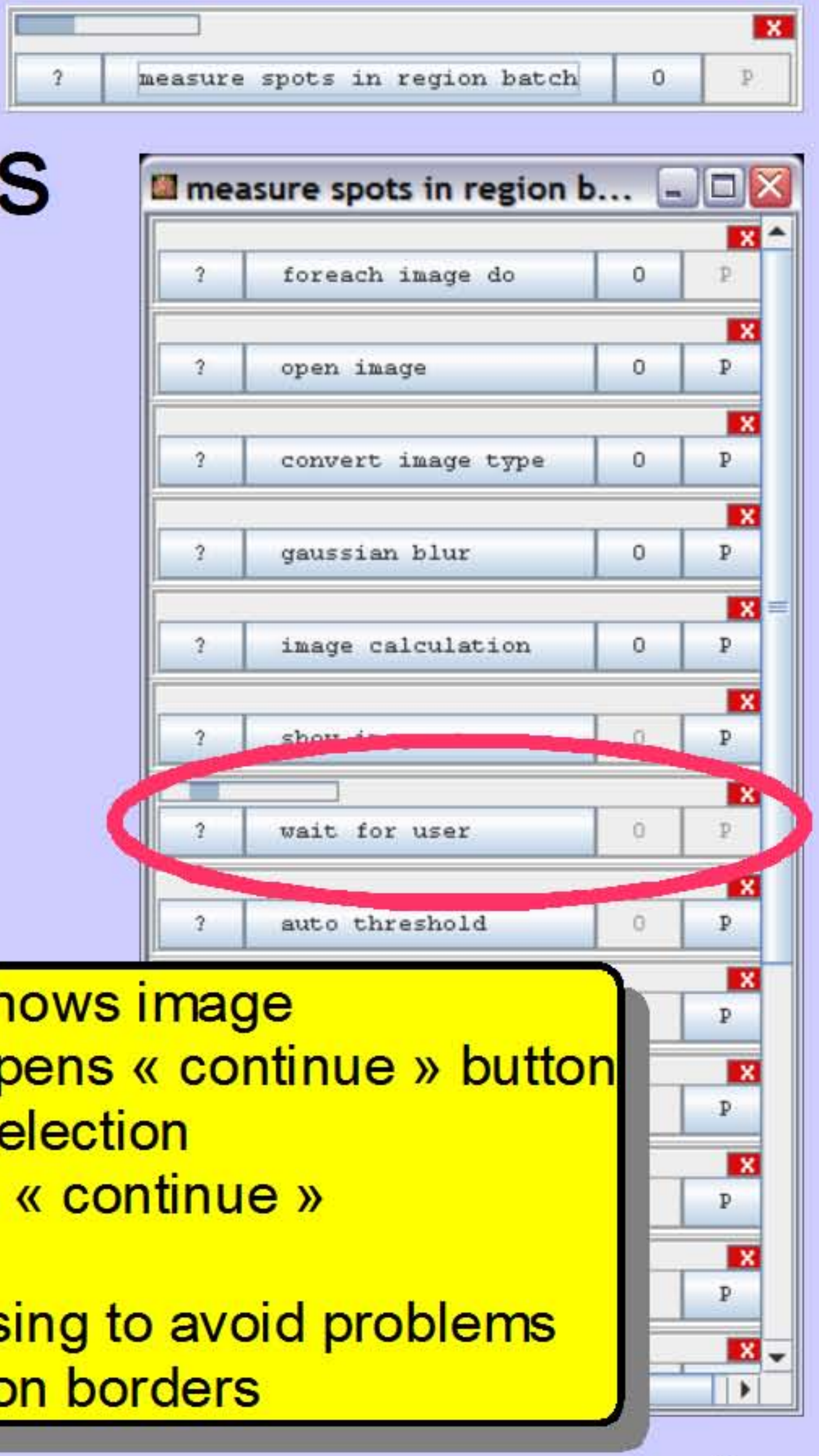


new requirement:

- count and measure plaques only for some selected cells

Measuring stained proteins

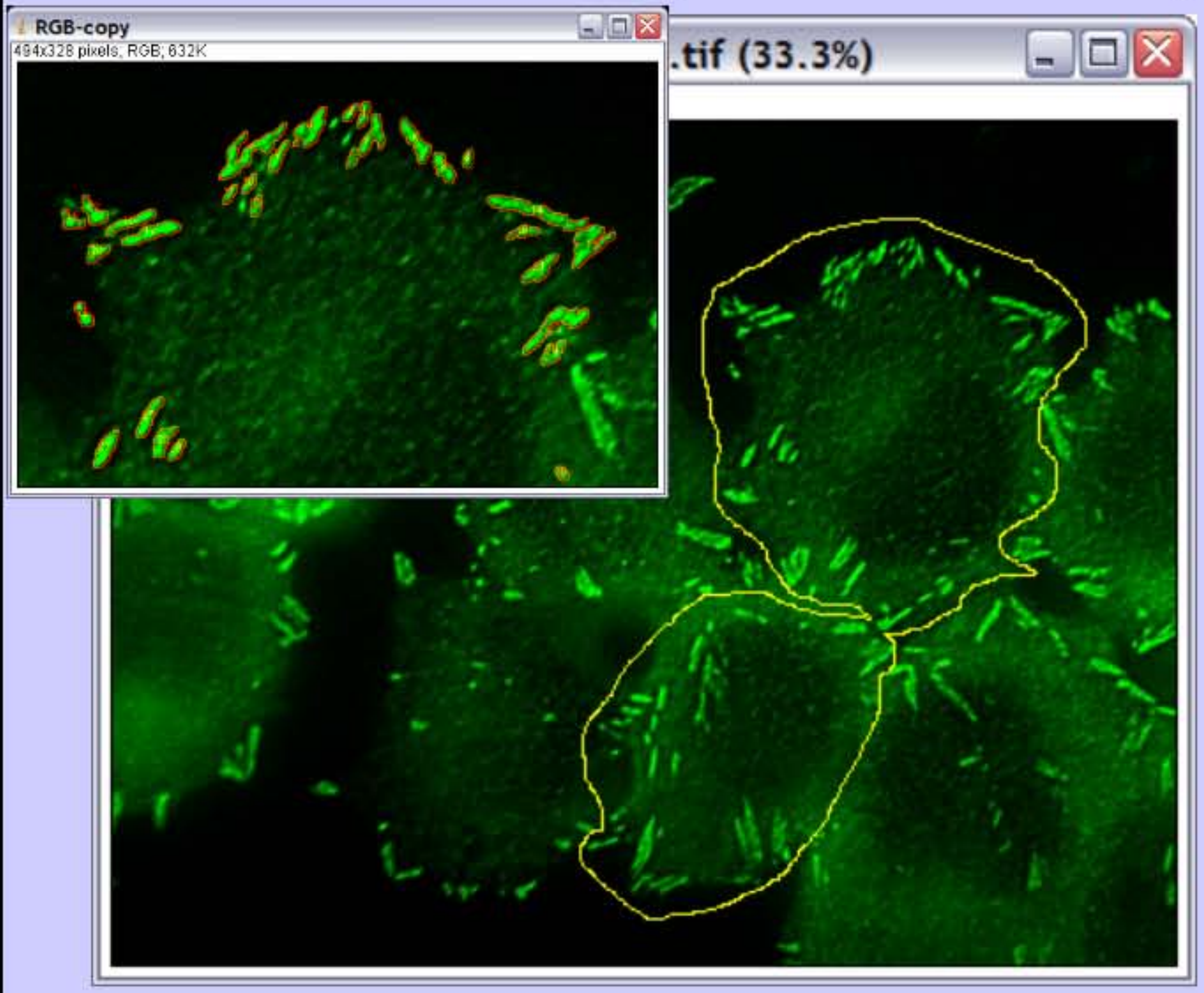


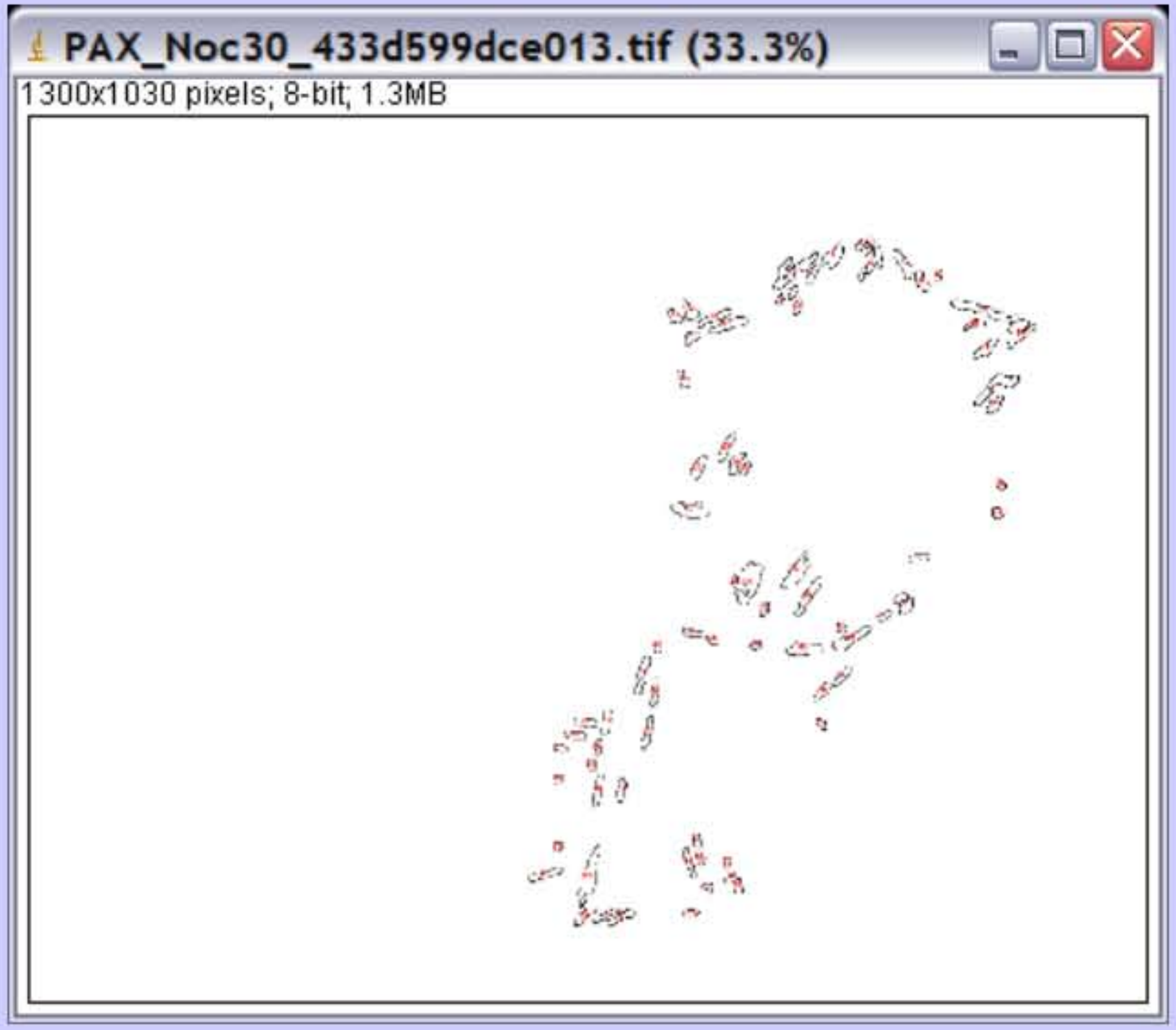


- application shows image
- application opens « continue » button
- user draws selection
- user presses « continue »

- extra processing to avoid problems at the selection borders

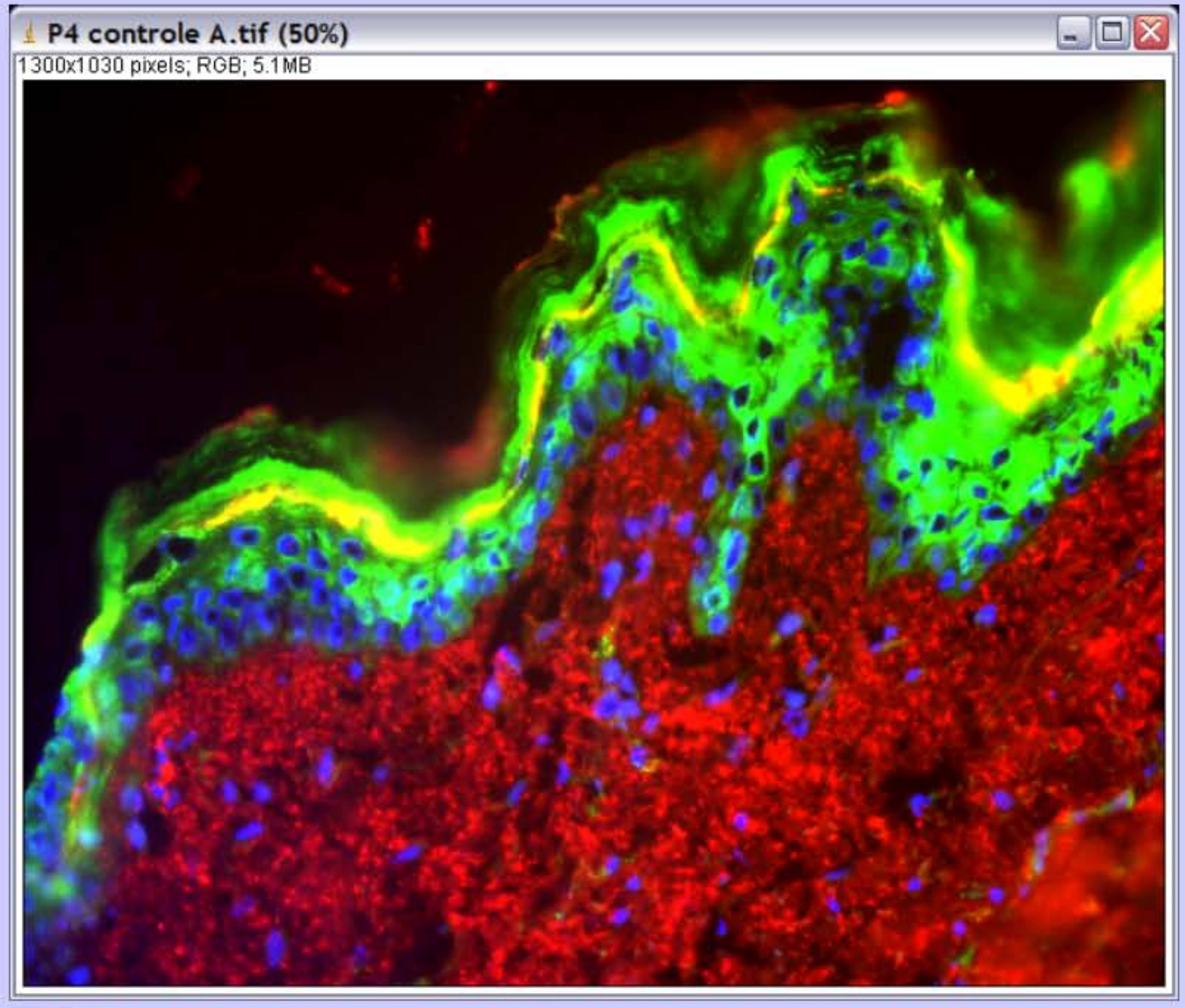
Measuring stained proteins




PAX_Noc30_433d599dce013.tif (33.3%)
1300x1030 pixels; 8-bit; 1.3MB

only the plaques within the selection are taken into account

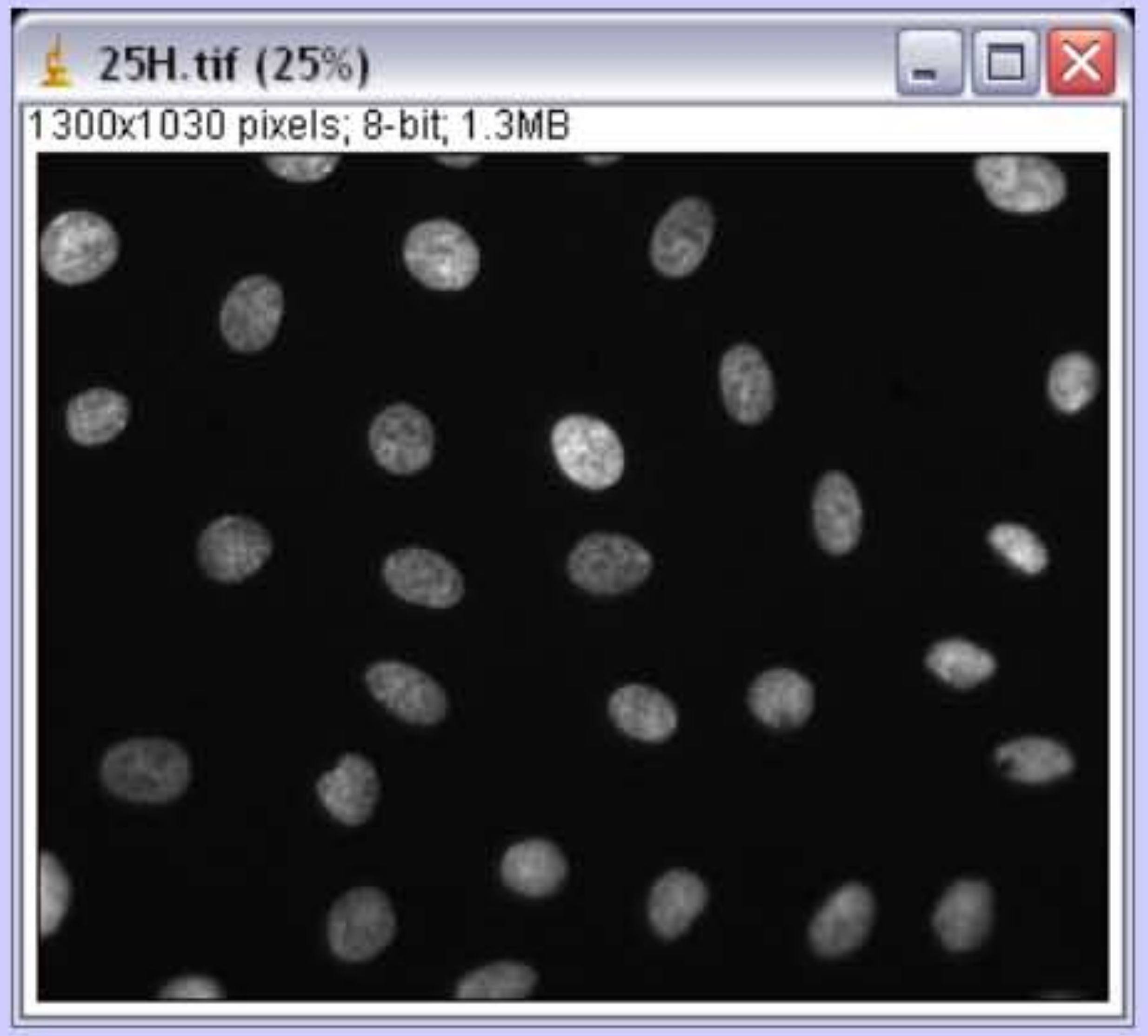
Counting nuclei

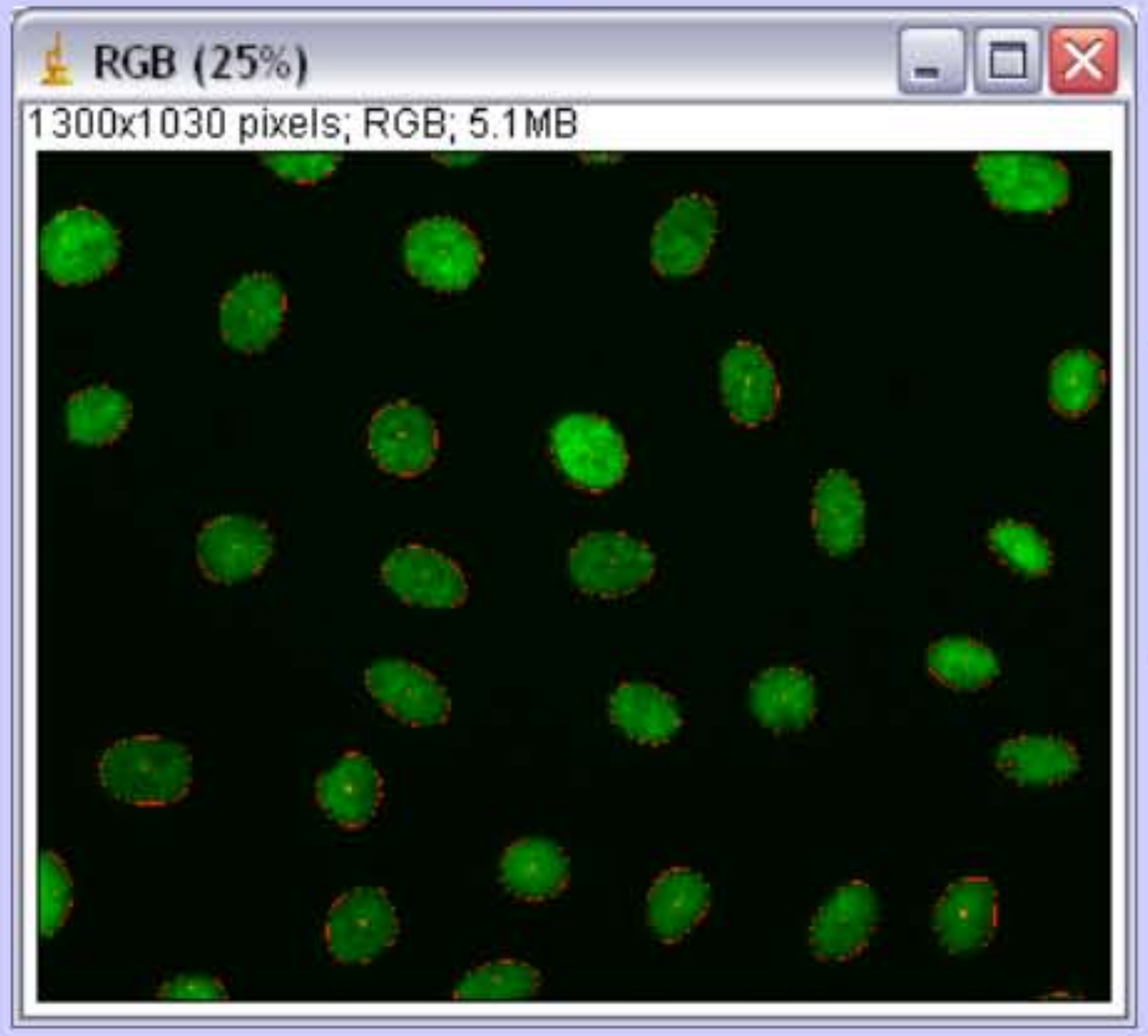


applications

- count cells or nuclei
- count stain combinations
  - blue only, red and blue, ...

Counting nuclei





How many cells are there?

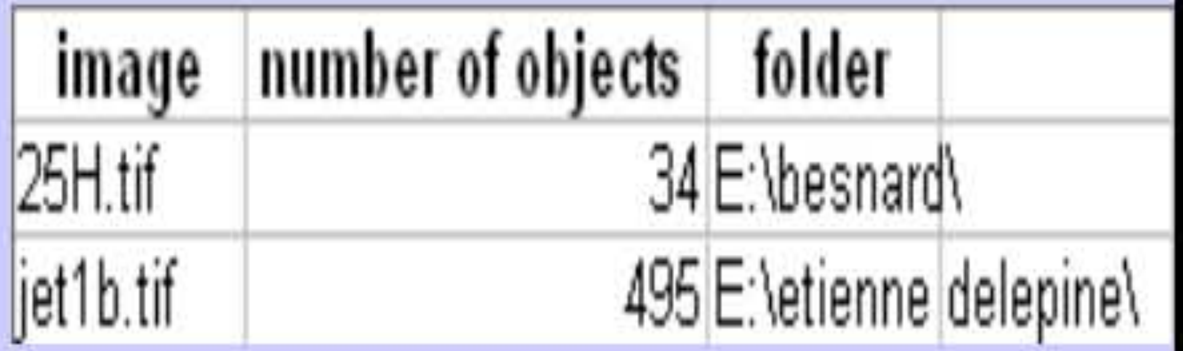
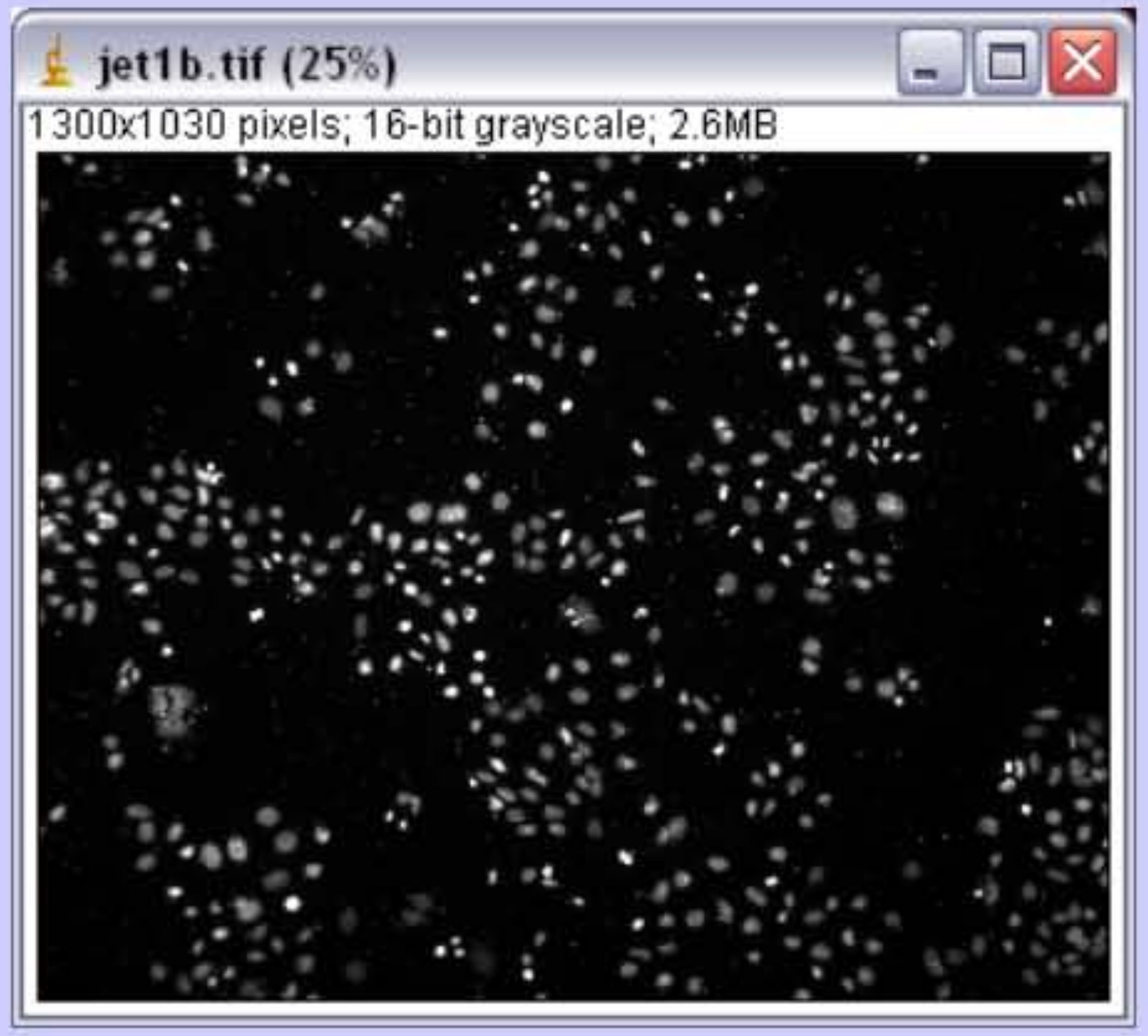


image	number of objects	folder
25H.tif	34	E:\besnard\
jet1b.tif	495	E:\etienne delepine\

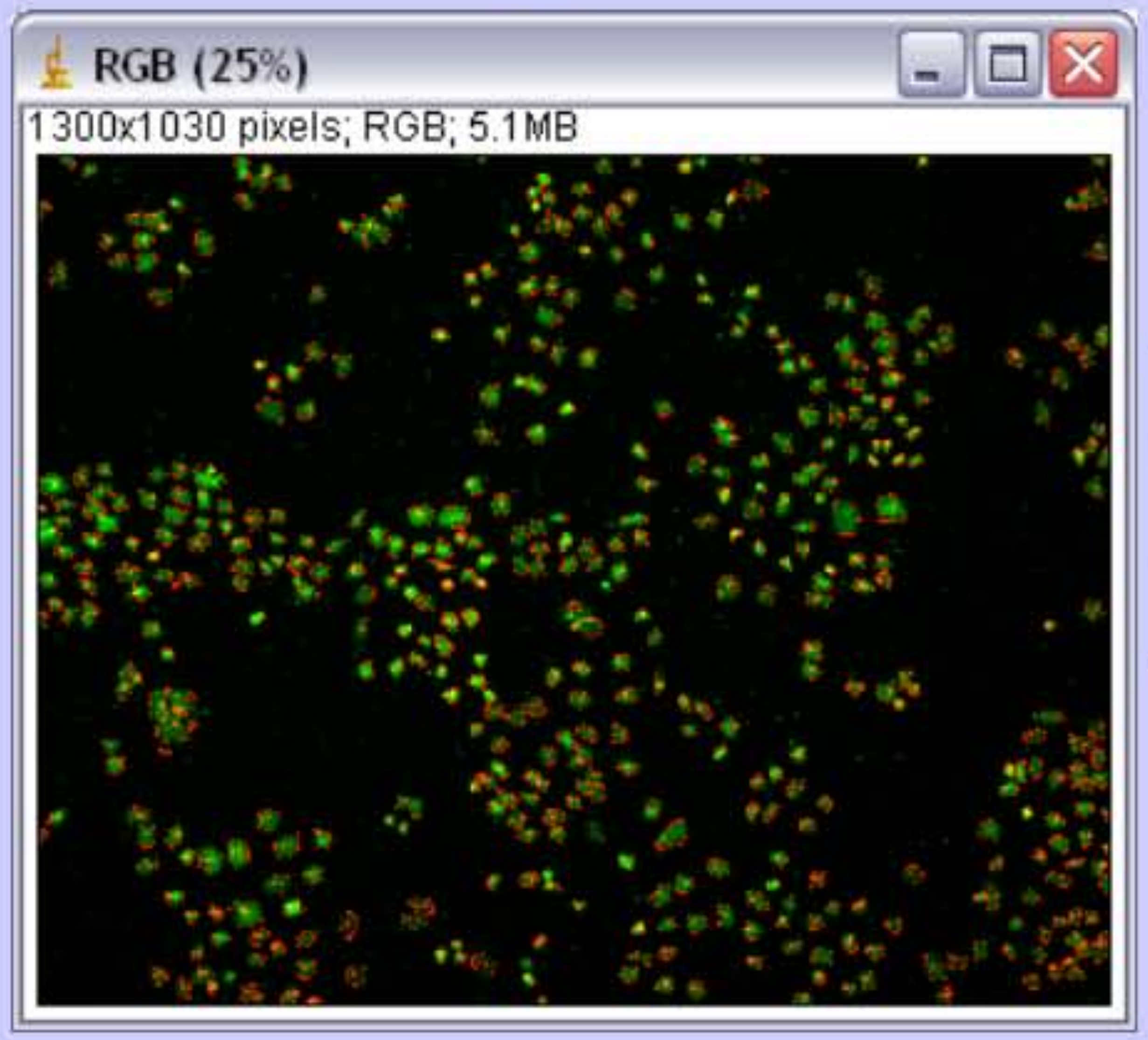
- watershed
- derivative
- local threshold

- full automatic
- interactive

Counting nuclei


jet1b.tif (25%)

1300x1030 pixels; 16-bit grayscale; 2.6MB


RGB (25%)

1300x1030 pixels; RGB; 5.1MB

How many cells are there?

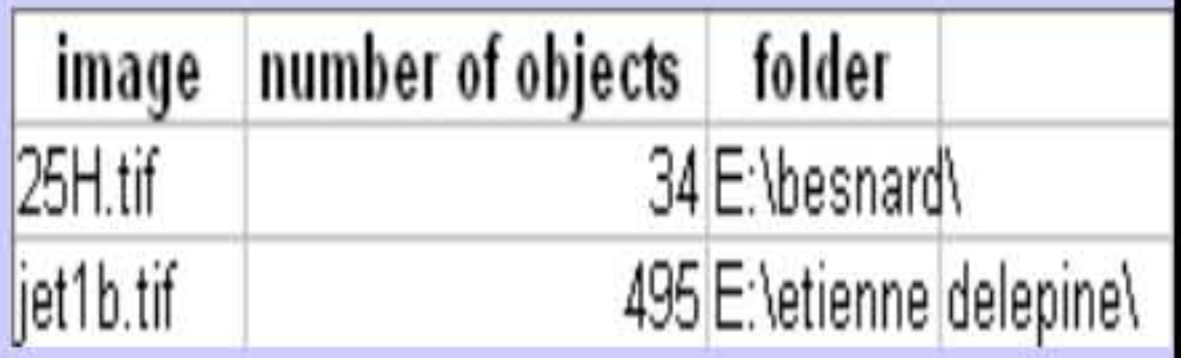
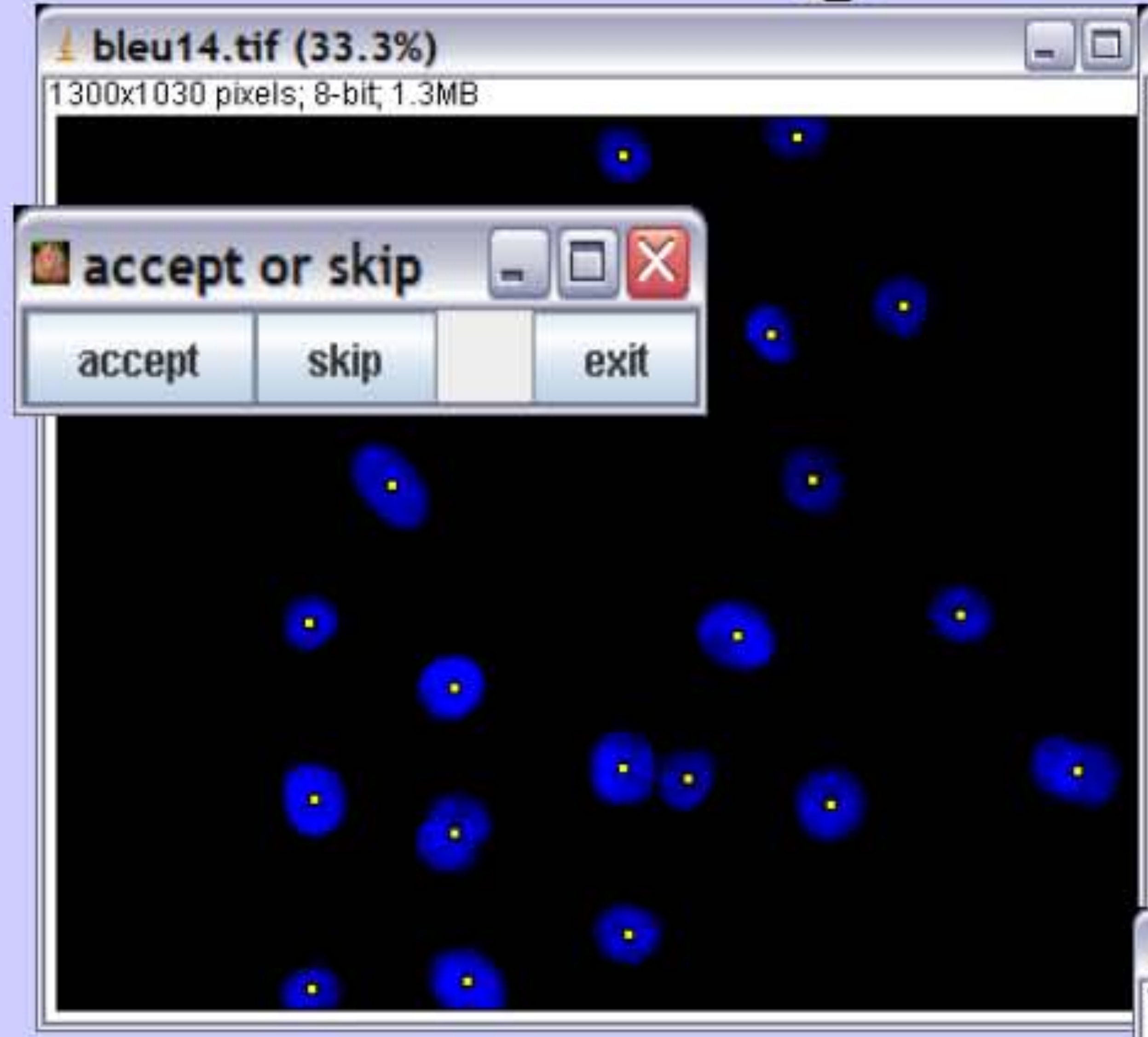


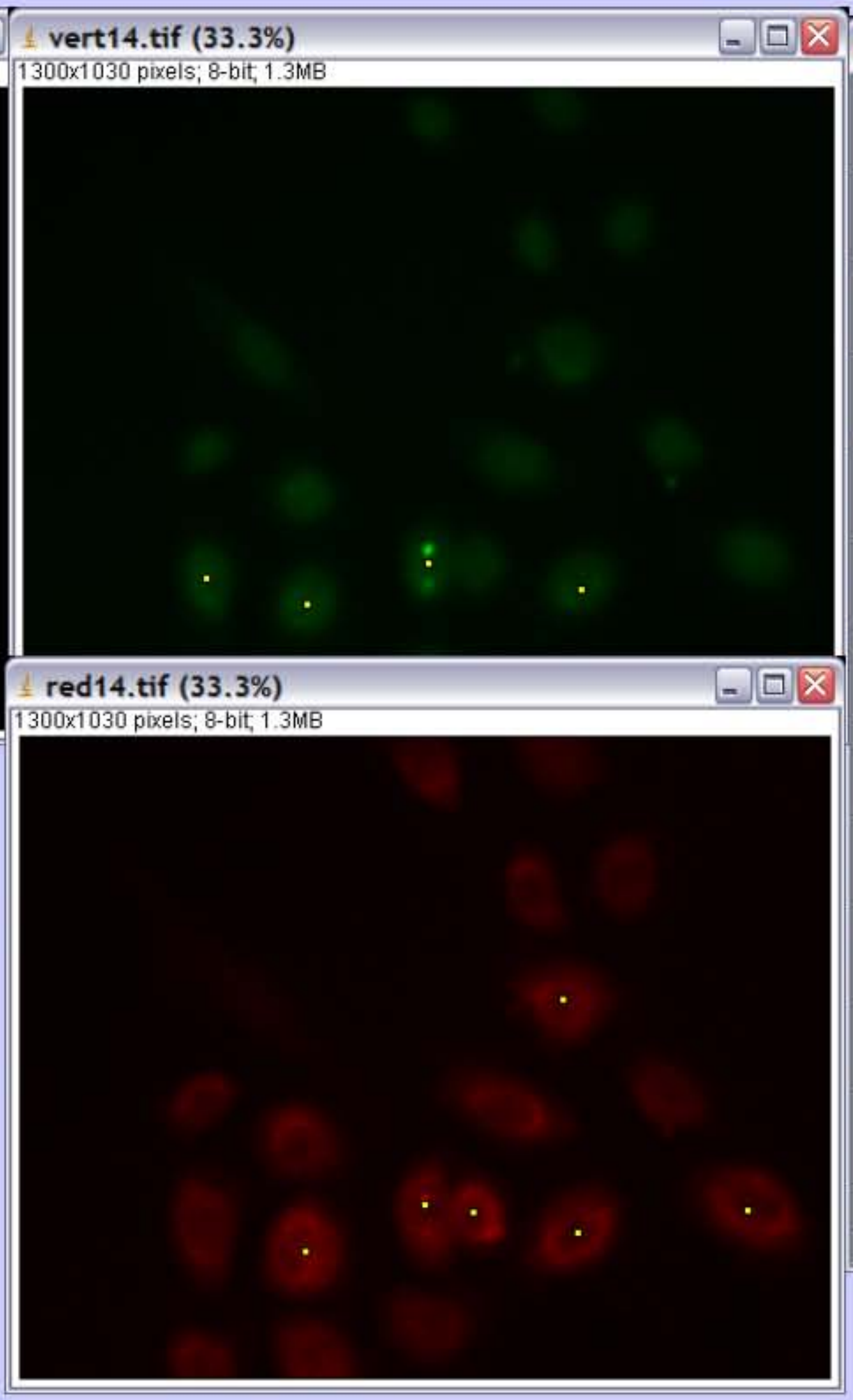
image	number of objects	folder
25H.tif	34	E:\besnard\
jet1b.tif	495	E:\etienne delepine\

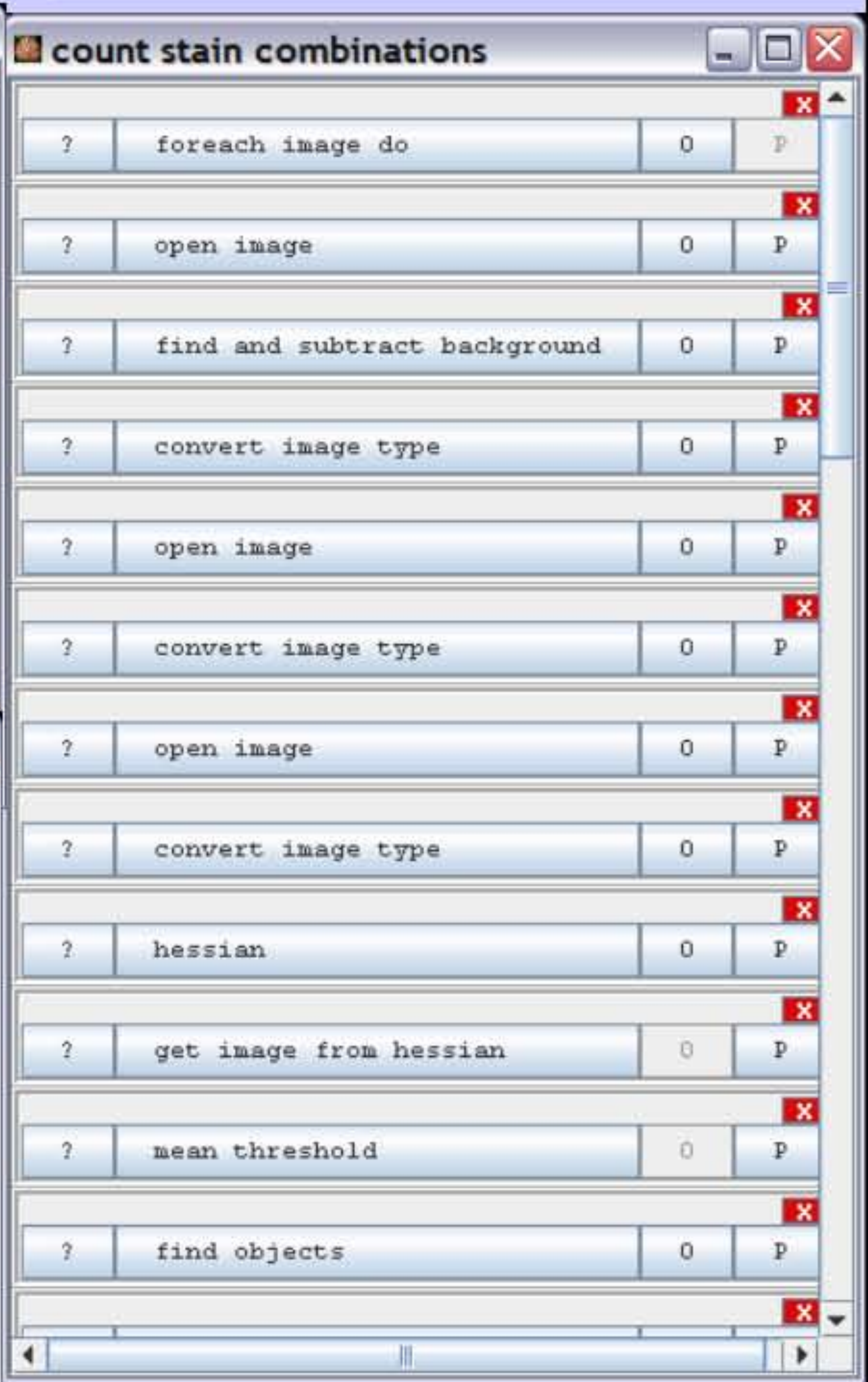
- watershed
- derivative
- local threshold

- full automatic
- interactive

Counting nuclei



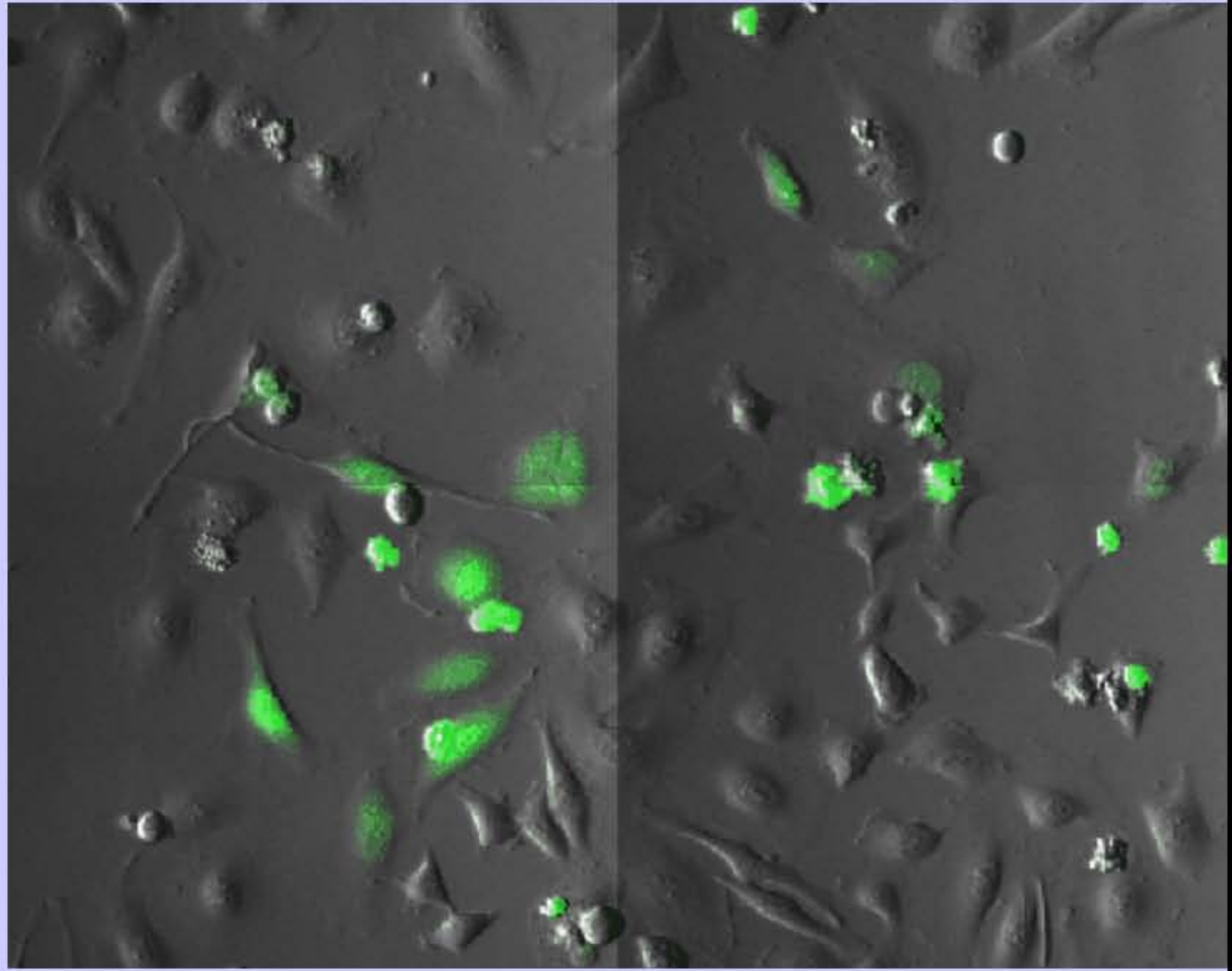

vert14.tif (33.3%)
1300x1030 pixels; 8-bit; 1.3MB
red14.tif (33.3%)
1300x1030 pixels; 8-bit; 1.3MB



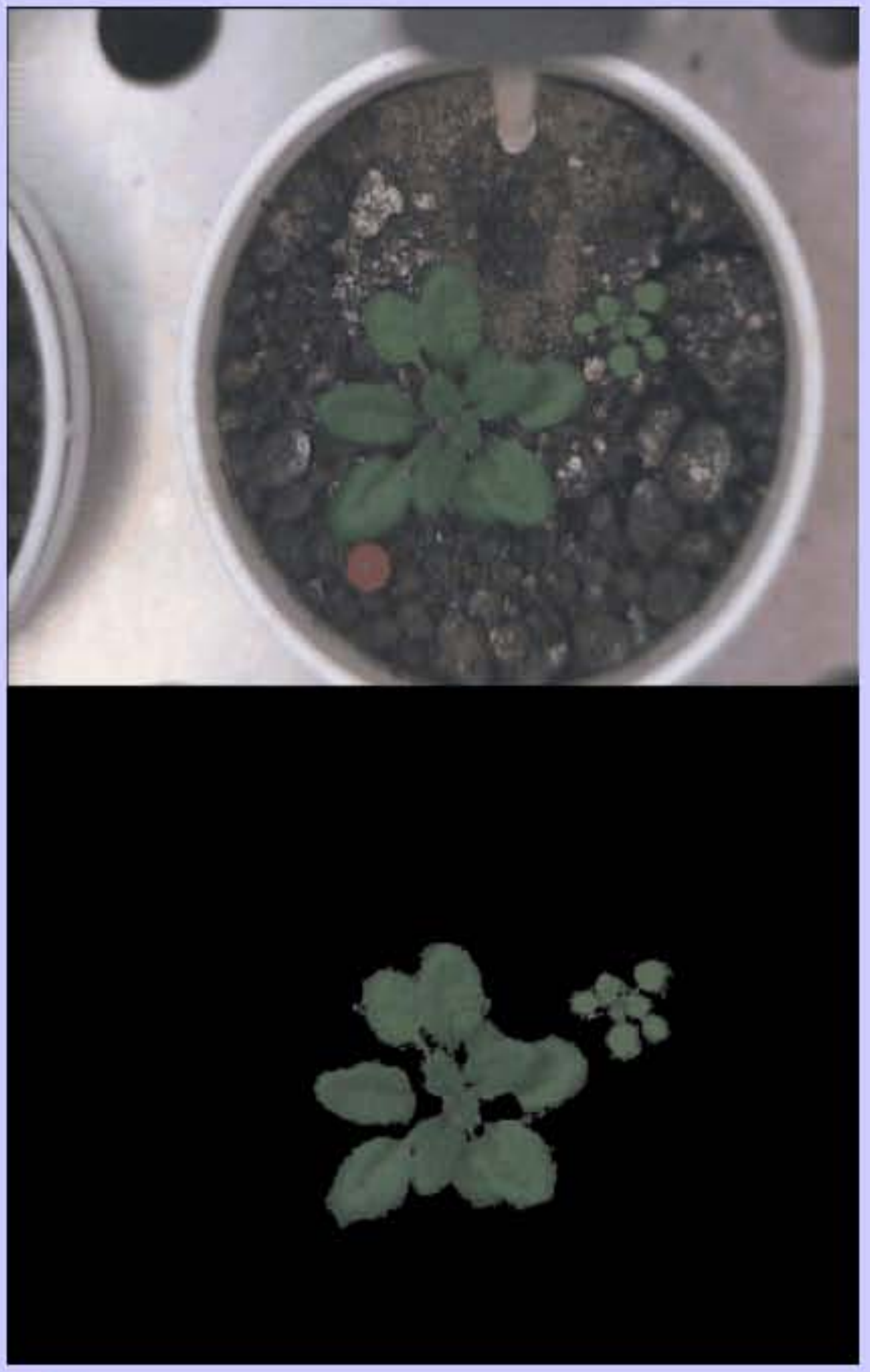
- interactive
  - application stops
  - user corrects point selections

Automation of simple tasks

- batch type conversions
- creating compressed movies from large data sets
  - use virtual stack opener + QuickTime Stack Writer plugin
- creating overlay of fluorescence and phase contrast image series
- ...



Further projects and projects in work



- measuring growth of plant surface (first version deployed)
- measuring growth of plant roots
- 3d modelling from histological sections
  - mouse
  - palm tree meristem

Conclusions

MRI Cell Image Analyzer

ImageJ + visual scripting

- rapidly
  - create image analysis applications
  - automate image analysis tasks
    - batch applications
    - semi-automatic applications
- interface application developer - scientist
  - easy to use by non computer experts
- the framework helps create new operations at the programming level
- all ImageJ methods can be accessed with the help of proxies
- used at Montpellier RIO Imaging
  - to create image analysis applications on demand
  - in close collaboration with the biologists
- made it possible to provide solutions that could not be obtained with standard image analysis packages
- outlook:
  - allow hierarchical applications (the use of applications in applications)
  - allow to work seamlessly with very big data sets (elaborate the virtual stack concept)

The last slide

- Thanks to
  - Wayne Rasband
  - all ImageJ plugin and macro authors
  - the ImageJ community
  - The ImageJ conference - organisation
  - Montpellier RIO Imaging
  - the research groups that participated in developing image analysis applications

Thank you, for your attention!

? Questions ?

References

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

- M. Boshernitsan, M. Downes
  Visual Programming Languages: A Survey. Technical Report CSD-04-1368
  University of California, Berkeley, 2004

- E. Gamma, R. Helm, R. Johnson and J. Vlissides
  Design Patterns: Elements of Reusable Object-Oriented Software
  Addison-Wesley, 207-, 1995

- I.R. Forman and N. Forman
  Java Reflection in Action
  Manning Publications, 2004

- E. Meijering, M. Jacob, J.-C. F. Sarria, P. Steiner, H. Hirling and M. Unser
  Design and Validation of a Tool for Neurite Tracing and Analysis in Fluorescence Microscopy Images
  Cytometry, 58A, 167-176, 2004

- S. Beucher
  The Watershed Transformation Applied to Image Segmentation
  Scanning Microscopy International, 6, 299-314, 1992