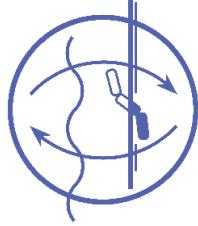


Look@NanoSIMS

A tool for the analysis of nanoSIMS data
in environmental microbiology

developed by Lubos Polerecky (2009-2011)

Citation: Polerecky et al. (2012) Look@NanoSIMS – a tool for the analysis of nanoSIMS data in environmental microbiology. *Environmental Microbiology*



Max Planck Institute for Marine Microbiology
Bremen, Germany

Motivation

Satisfy the (diverse) needs of environmental microbiologists using nanoSIMS

- Analysis of many cells
 - rapid and reproducible definition
 - statistical analysis (correlations, comparison of cells and cell populations)
- The use of external information to guide nanoSIMS analysis
 - e.g., FISH, SEM, TEM, AFM images
- “Special requests”
 - Need for freedom

Result

A comprehensive tool for nanoSIMS data analysis

- Not only for microbiologists but also for scientists in any other discipline
- Open-source (Matlab)
- Being actively developed
- Free

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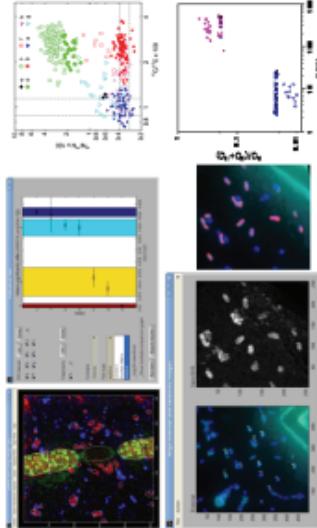


Start Micro-profiling Imaging Support Contact

Show pagesource Old revisions

Look@NanoSIMS

Look@NanoSIMS is a Matlab-based freeware program for the analysis of nanoSIMS data. It is an alternative to other programs developed for that purpose, such as WinImage, Open_MIMS or L'Image.



The screenshot shows the software's main interface with several windows. One window displays a scatter plot of mass-to-charge ratio (m/z) versus intensity. Another window shows a grayscale image of a sample area with overlaid color-coded regions. A third window shows a zoomed-in view of the sample with specific features highlighted. The interface includes various toolbars and menus typical of scientific data analysis software.

Table of Contents ▲

- Look@NanoSIMS
- Download
- Citation
- Features
- Contact
- Report a bug

Download

1. program: [version 2011-11-11](#)
2. manual: [download](#)
3. article in Environmental Microbiology: [download](#)
4. poster: [download](#)
5. powerpoint presentation with a brief overview of the program: [download](#)
6. example datasets for testing: raw data ([download](#)), processed data ([download](#))
7. example of the summary file outputG.pdf: [\(download\)](#)
8. everything: [link](#)

9. you can also join a dedicated Look@NanoSIMS group at [Dropbox](#) to access the most up-to-date version of the program. Just install Dropbox and let us know your e-mail address, so that it can be added to the group.

How to use the program: details in...

The screenshot shows a software application window titled "Look@NanoSIMS*". The main content area displays a hierarchical table of contents for the user manual. The tree structure includes:

- Installation instructions
 - Installation under Microsoft Windows
 - Installation under Linux
 - Installation under Mac OS X
- Updating the program
- Adjusting program appearance
 - Adjusting graphical output appearance
- Running Look@NanoSIMS
 - Organization of the input and output data
- A typical data processing session
 - Analysis of a single dataset—"from scratch"
 - Analysis of a single dataset—continuation or modification of previous
- Analysis of multiple datasets
 - Data processing functions of Look@NanoSIMS
 - Loading nanoSIMS dataset from disk
- Display of mass images
 - Drift-corrected accumulation of planes
 - Display of accumulated mass images
- Graphical output I
 - ROI definition
 - Text output
- Graphical output II
 - ROI classification
 - Statistical comparison of ROIs and ROI classes
 - Metafile processing

At the top right of the window, there is a note: "Lubos Polerecky† Max-Planck Institute for Marine Microbiology, Bremen, Germany version 11-11-2011". Below the table of contents, the URL <http://www.microsen-wiki.net/doku.php?id=lans> is displayed.

Summary

Look@NanoSIMS is a program for the analysis and processing of nanoSIMS and SIMS data. It can be used freely for research purposes. It is an open-source program, and can therefore be modified freely by anyone to meet their specific needs. We ask users who make modifications to the program to share them with others by e-mailing their improved code to the person in charge of the program maintenance, who will then post the updated version on the program's website (currently: LP). If you experience problems with the execution of the program, discuss them first with your colleagues that are experienced in using the program. If this does not solve your problem, contact

How to interpret the results: details in...

environmental
microbiology

Environmental Microbiology (2012)

doi:10.1111/j.1462-2920.2011.02681.x

Look@NanoSIMS – a tool for the analysis of nanoSIMS data in environmental microbiology

Lubos Polerecky,* Birgit Adam, Jana Milucka,
Niculina Musat, Tomas Wagner and
Marcel M. M. Kuypers
Max-Planck Institute for Marine Microbiology,
Celsiusstrasse 1, 28359, Bremen, Germany.

Introduction

Nanometre-scale secondary ion mass spectrometry (nanoSIMS) allows quantitative analysis of elemental and isotopic composition of a sample with a submicrometre spatial resolution, and has been applied in diverse

nanoSIMS fields ranging from ancient and modern marine

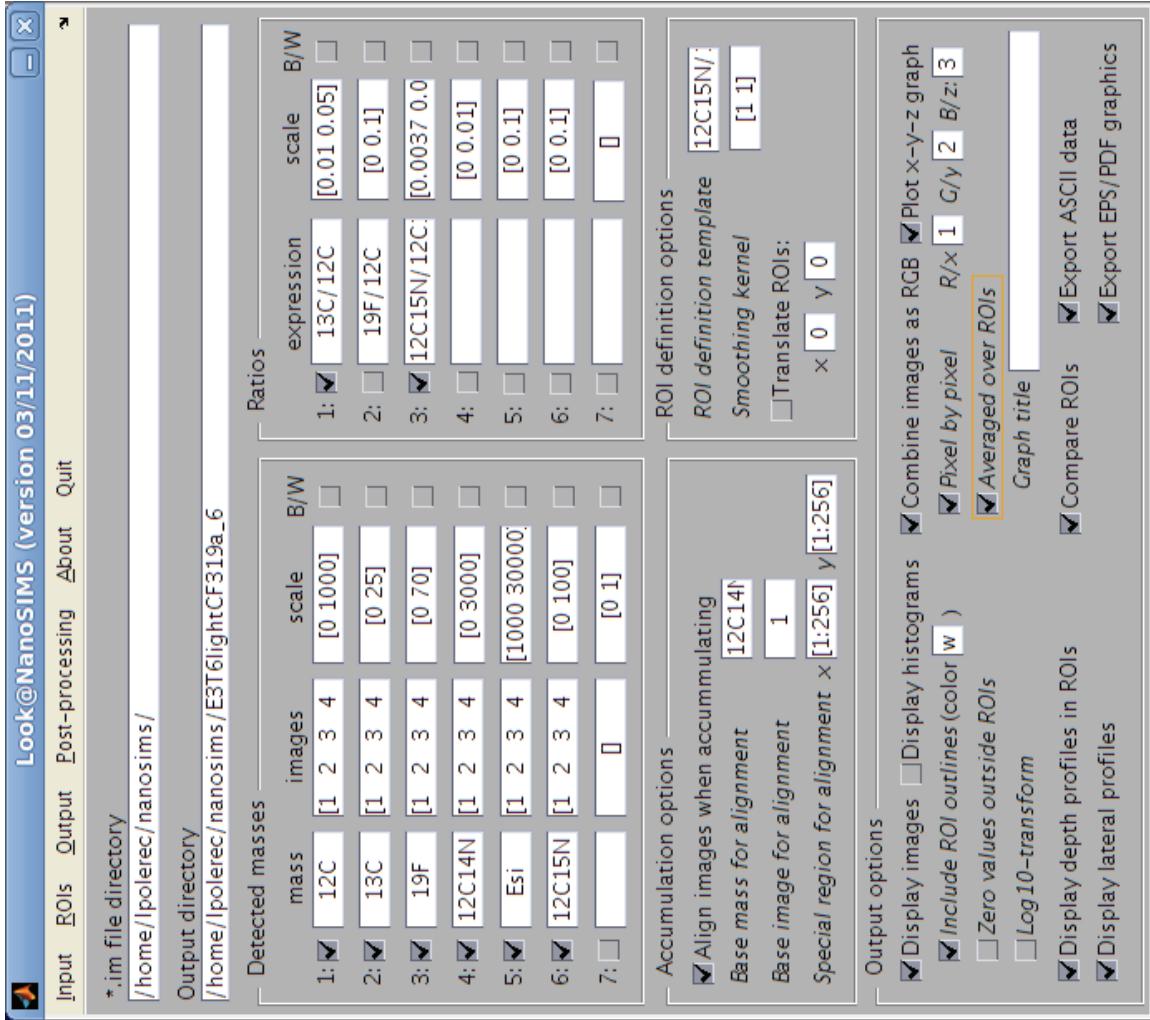


Comparison of features

Feature / Function	Look@NanoSIMS	Open_MIMS	WinImage	L'Image
dead time and QSA correction	+	-	+	+
Display of scanned planes	+	+	+	+
Drift-corrected accumulation	+	+	+	+
ROI definition				
manual	+	+	+	+
Semi-automated (interactive thresholding) based on an external image	+	+	-	-
ROI classification				
manual	+	+	-	-
automated				
Quantification of elemental and isotopic compositions				
images	+	+	+	+
histograms	+	+	-	+
depth profiles	+	+	+	+
lateral profiles	+	+	+	+
averages in ROIs	+	+	+	+
scatter plots of averages in ROIs [†]	+/-	+	+	+/-
dead time and QSA correction	-/-	+	+	+
δ-notation	-	-	-	-
arbitrary expressions	+	+	-	-
RGB composition	+	+	-	-
Image stitching	-	-	-	+
Statistical comparison of isotopic compositions				
in ROIs [†]	+/-	-/-	-/-	-/-
in ROI classes [†]	+/-	-/-	-/-	-/-
Open Source Platform Availability				
Matlab 2010b [‡] (multiplatform)	+	+	-	-
Free (MPI Bremen)	Free (NRIMS Harvard)	Aphelion (Windows XP [‡])	PV-WAVE (Windows XP [‡])	PV-WAVE (Windows XP [‡])
		commercial (Cameca)	commercial (L.R. Nittler)	commercial (L.R. Nittler)

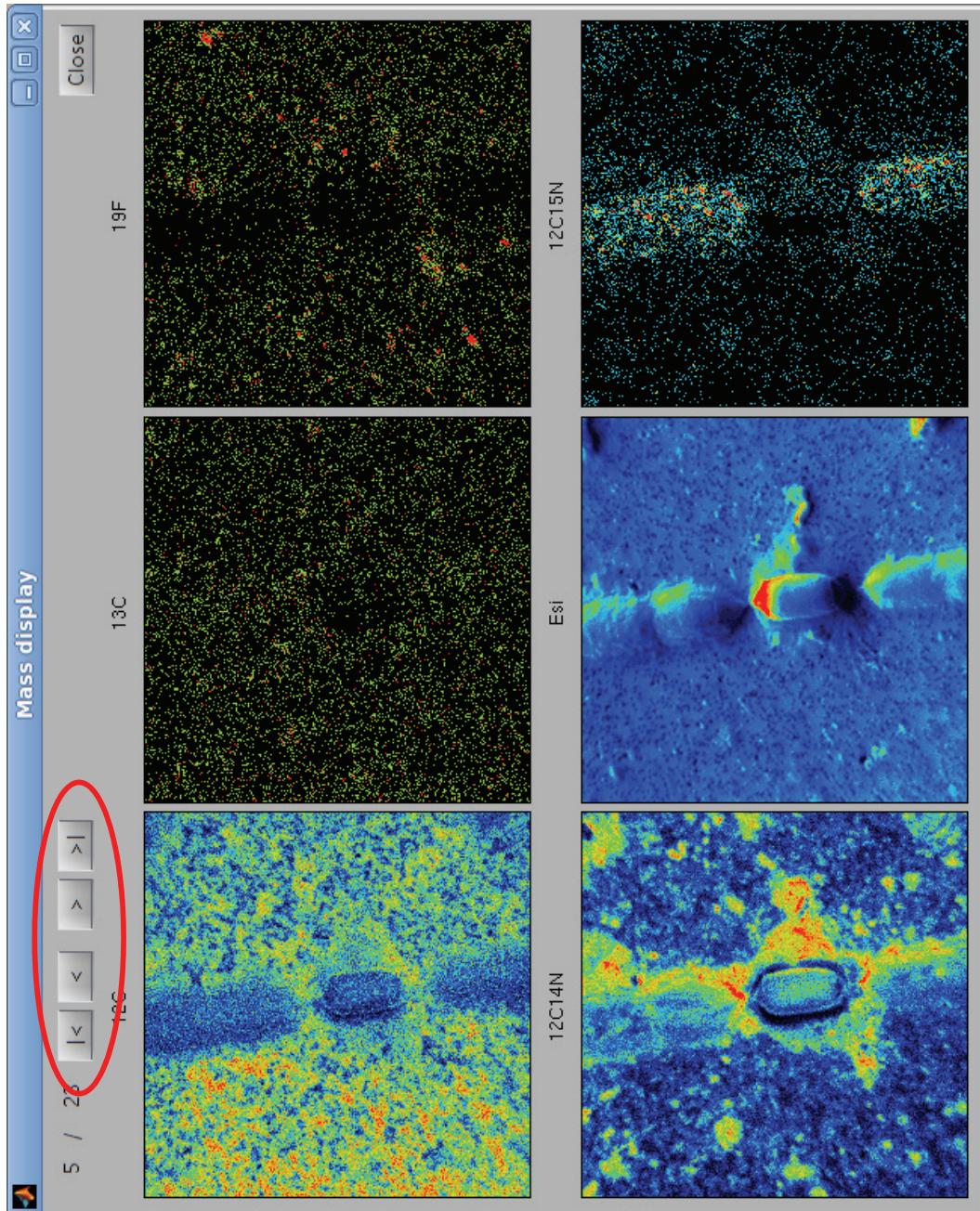
[†]single/multiple datasets
[‡]minimum version requirement

Graphical user interface



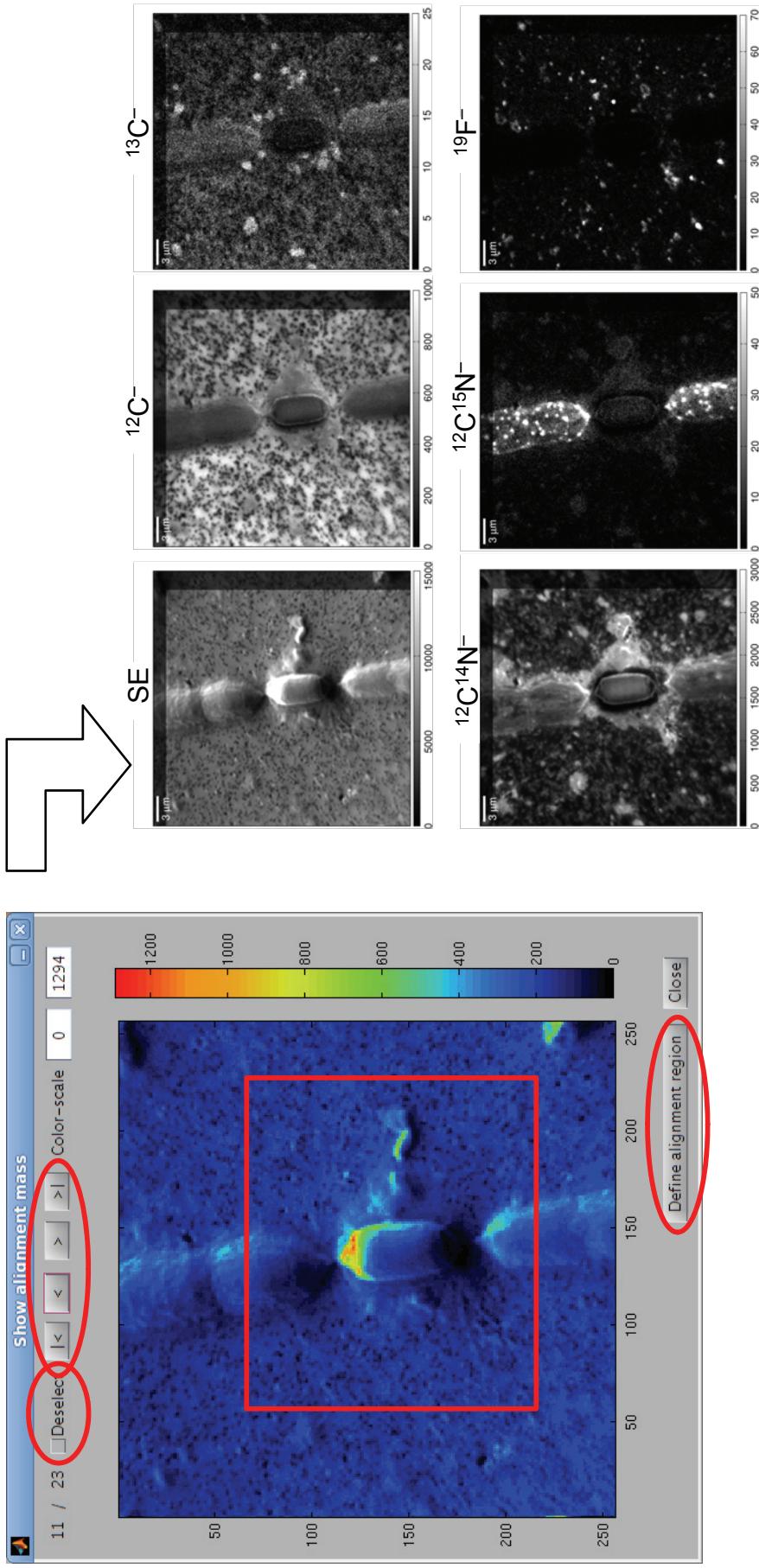
Common features

- Display of planes for all masses



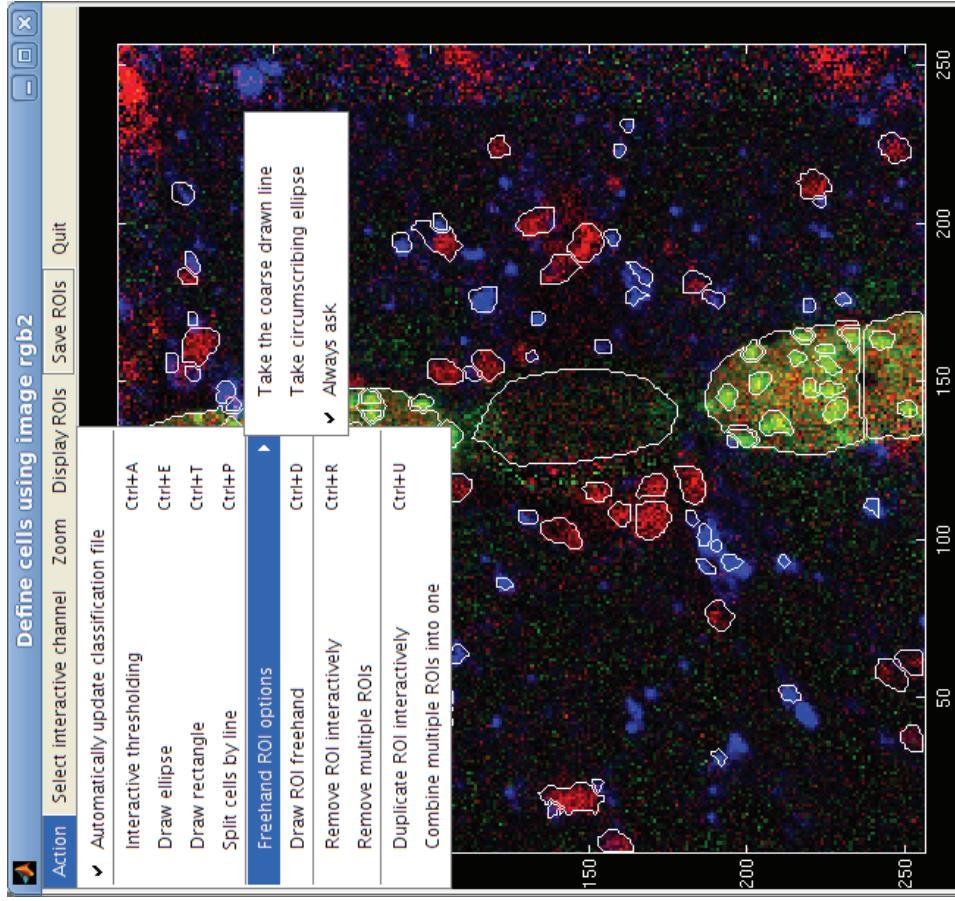
Common features

- Drift-corrected image accumulation



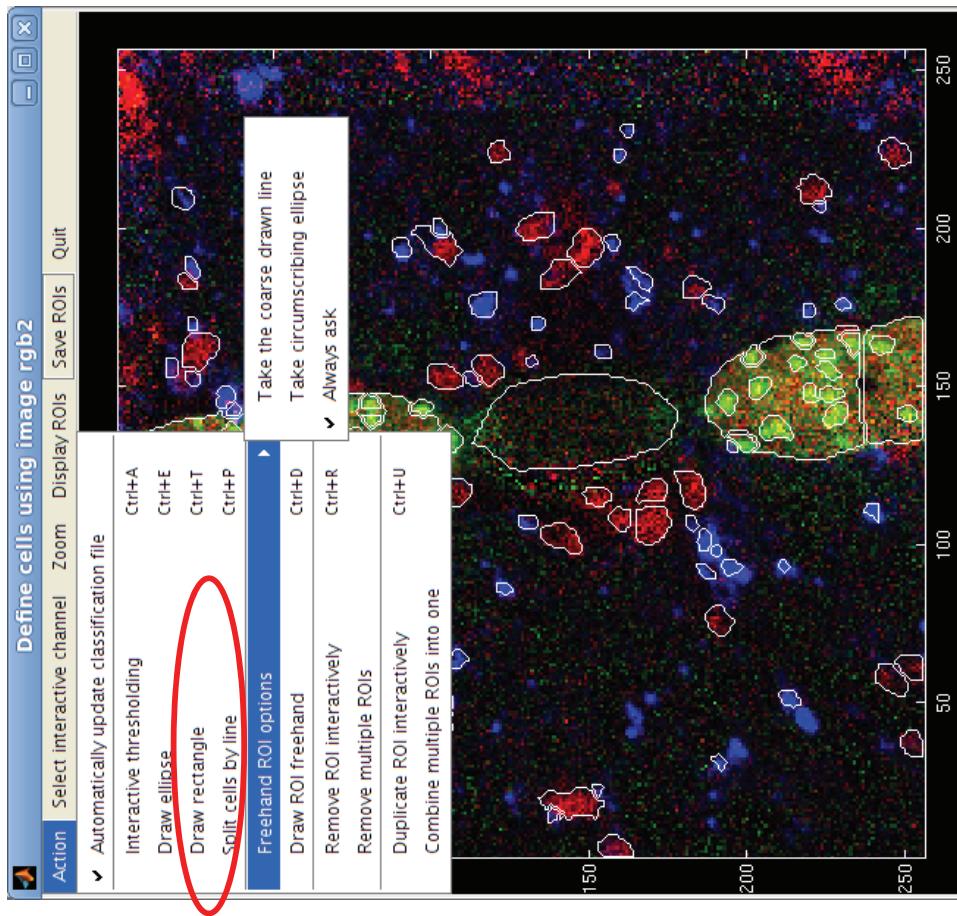
Common features

- ROI definition



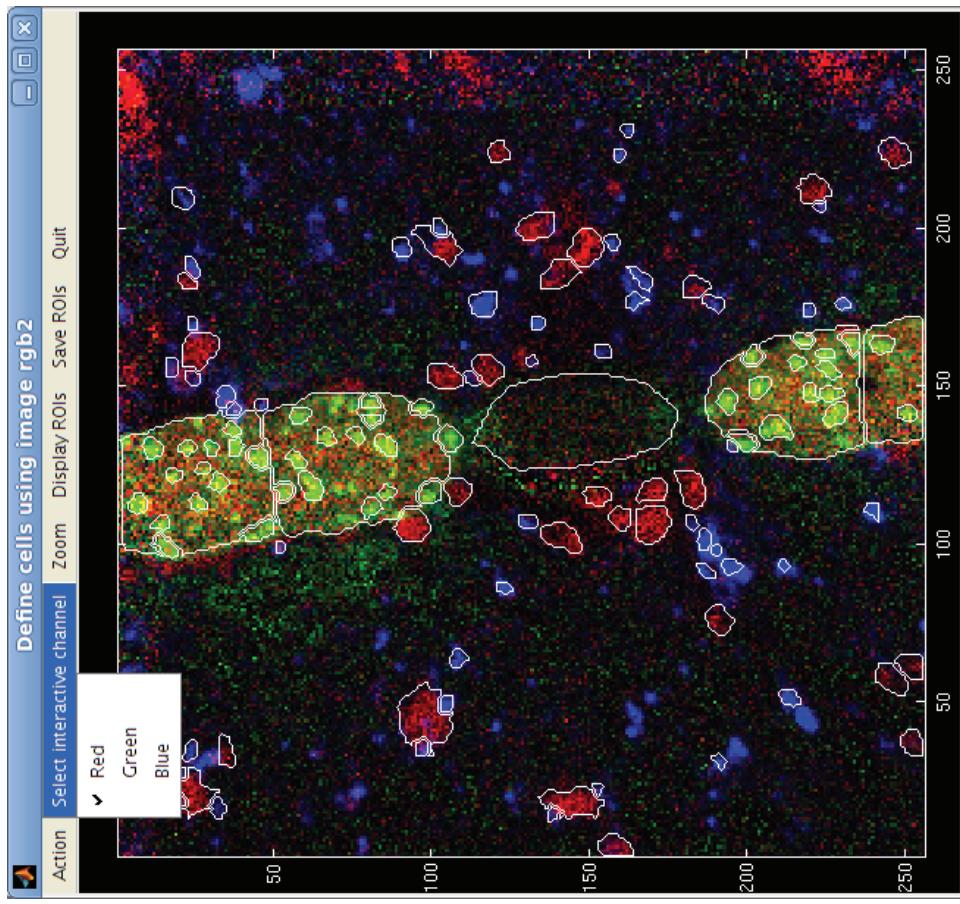
Common features

- ROI definition



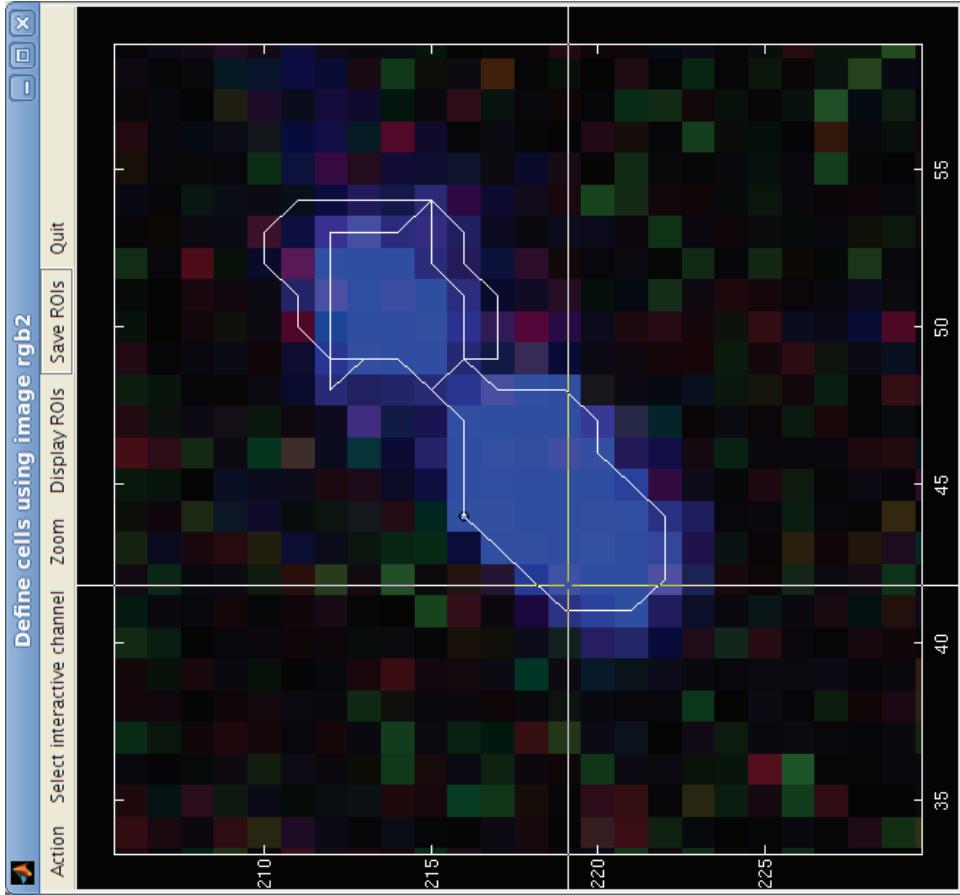
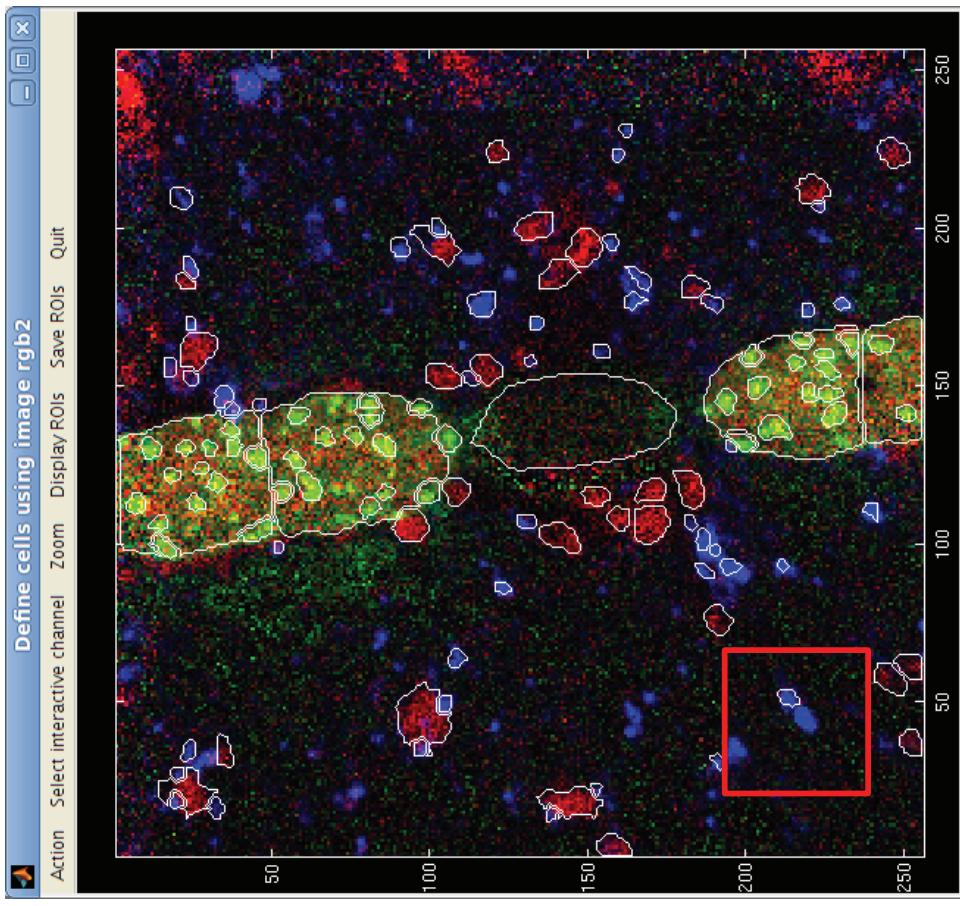
Common features

- ROI definition



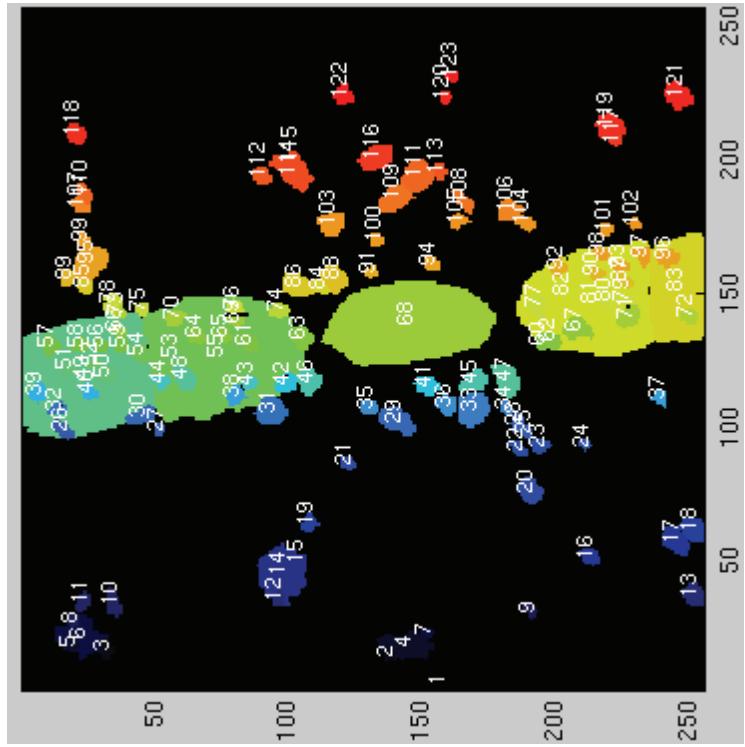
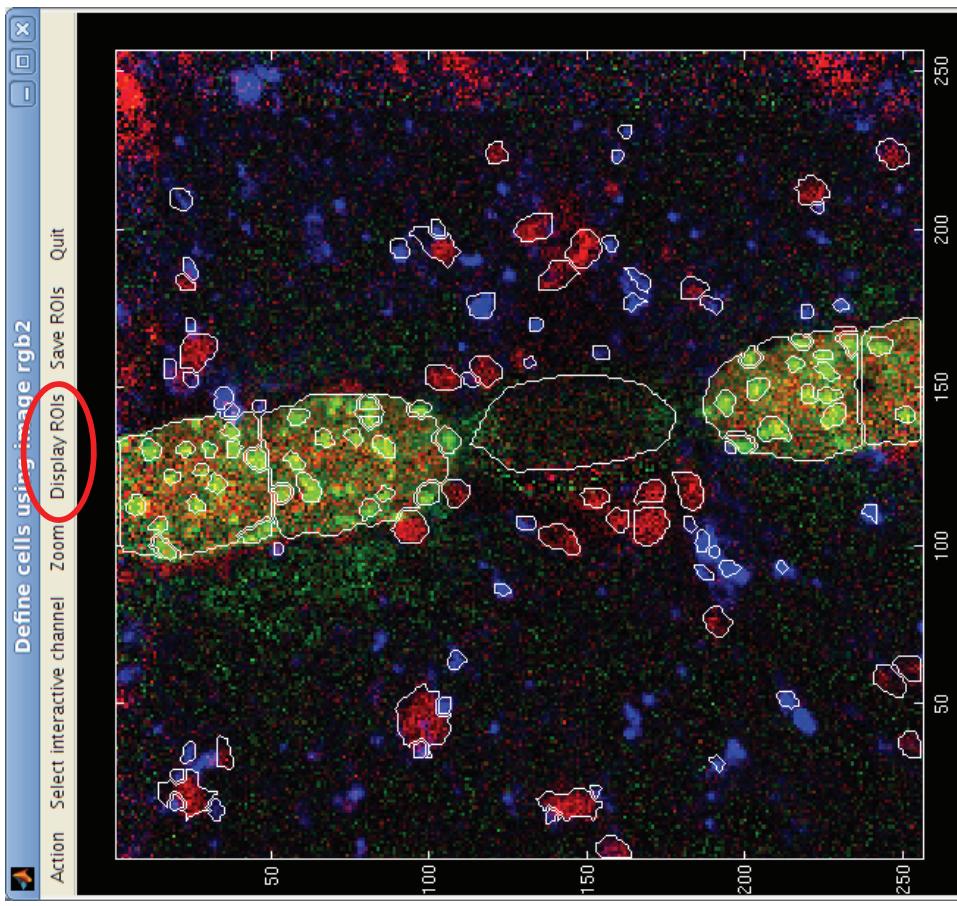
Common features

- ROI definition



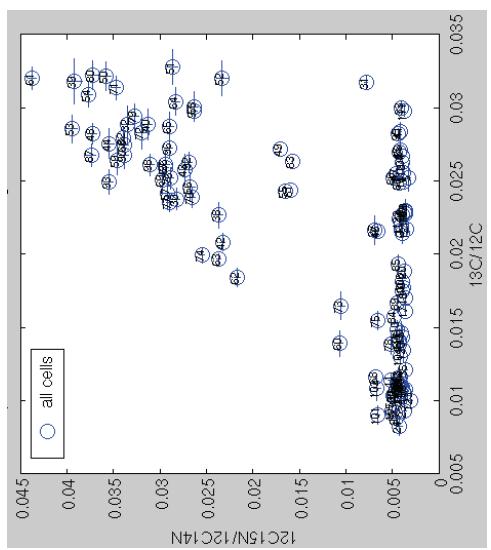
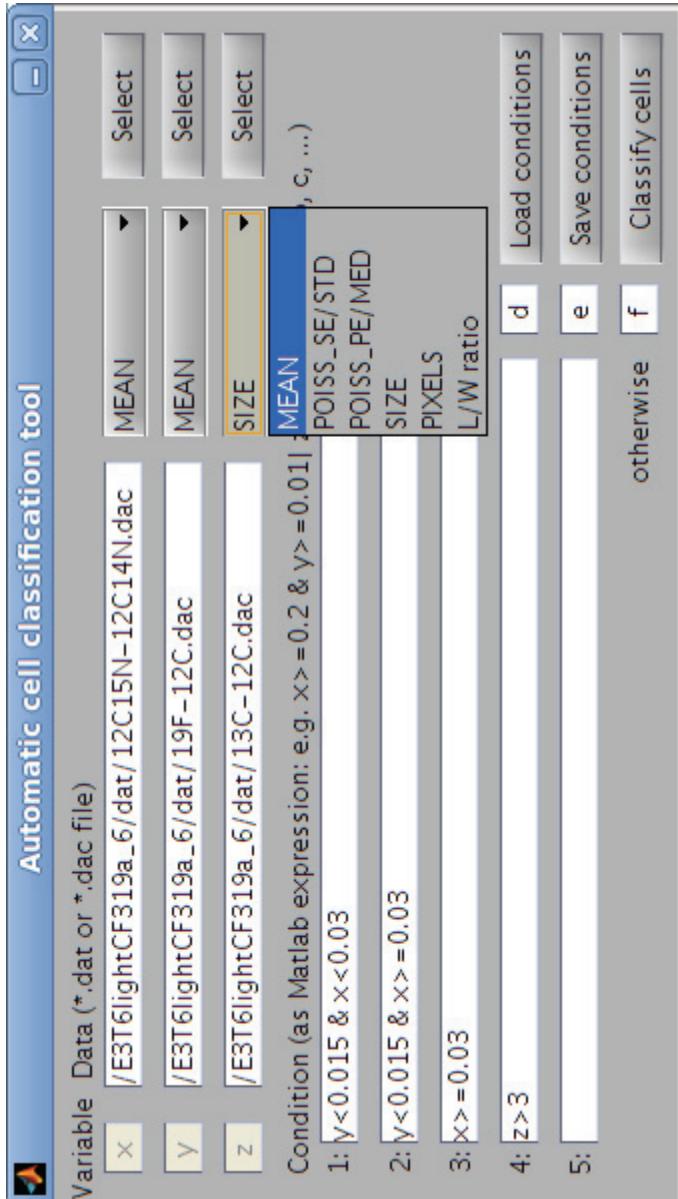
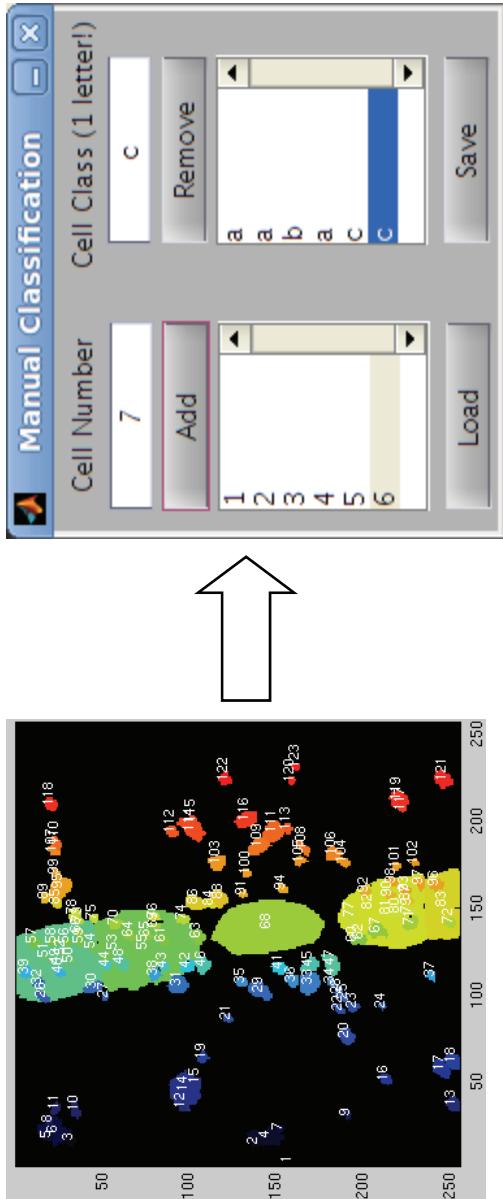
Common features

- ROI definition



Common features

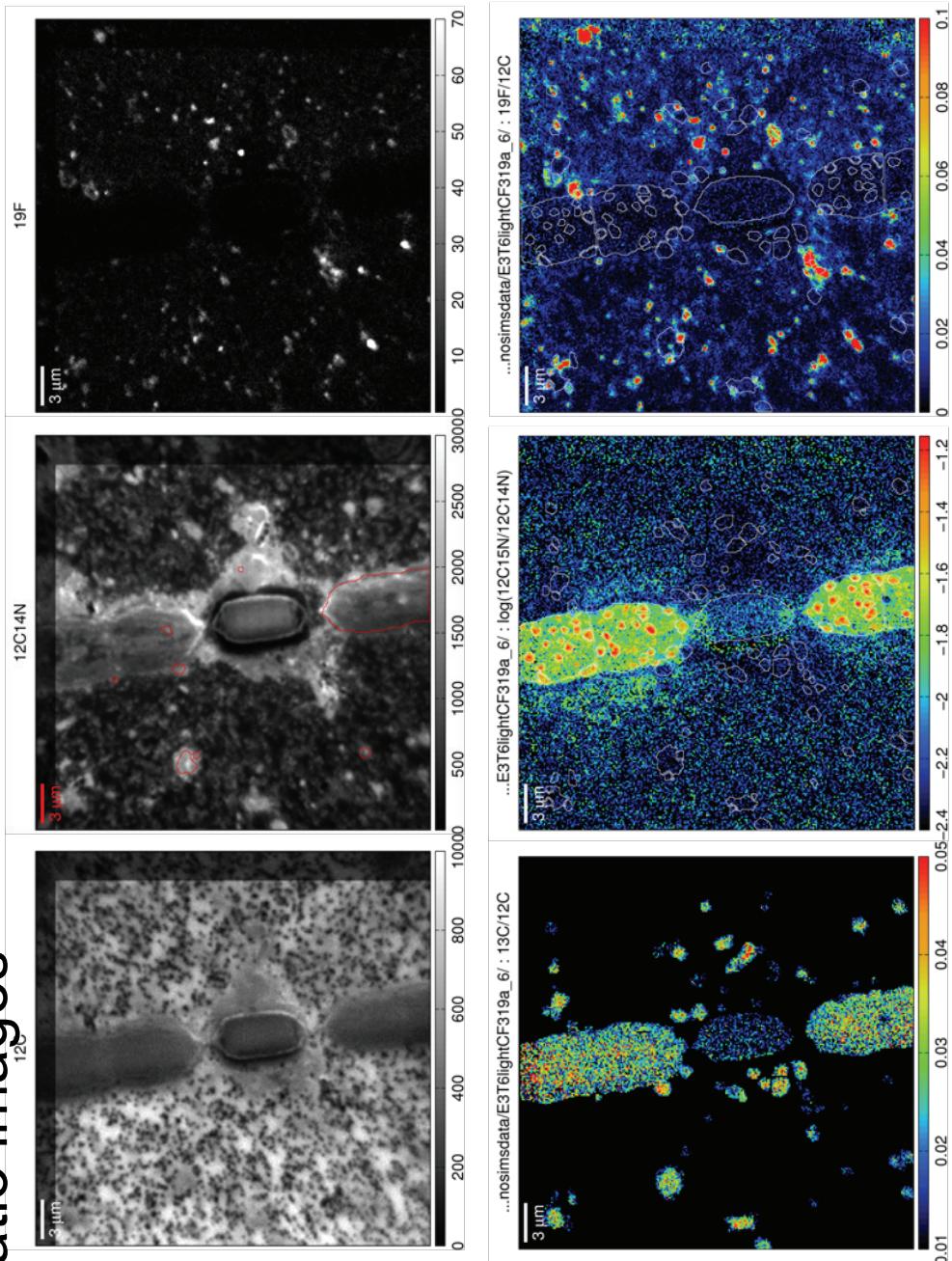
- ROI classification
 - Manual
 - Automated



Common features

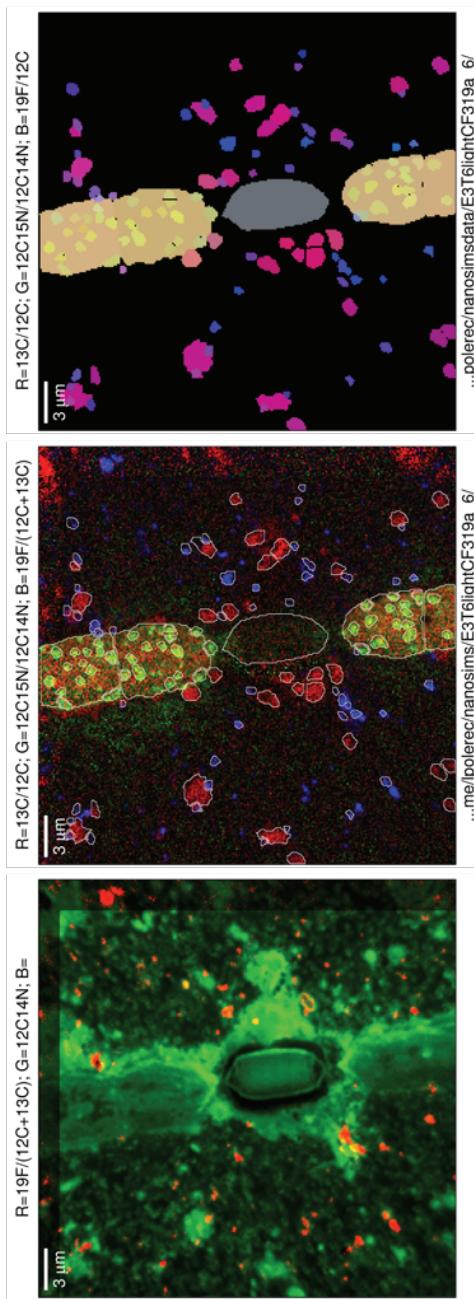
- Display and export of results

- Mass & Ratio images



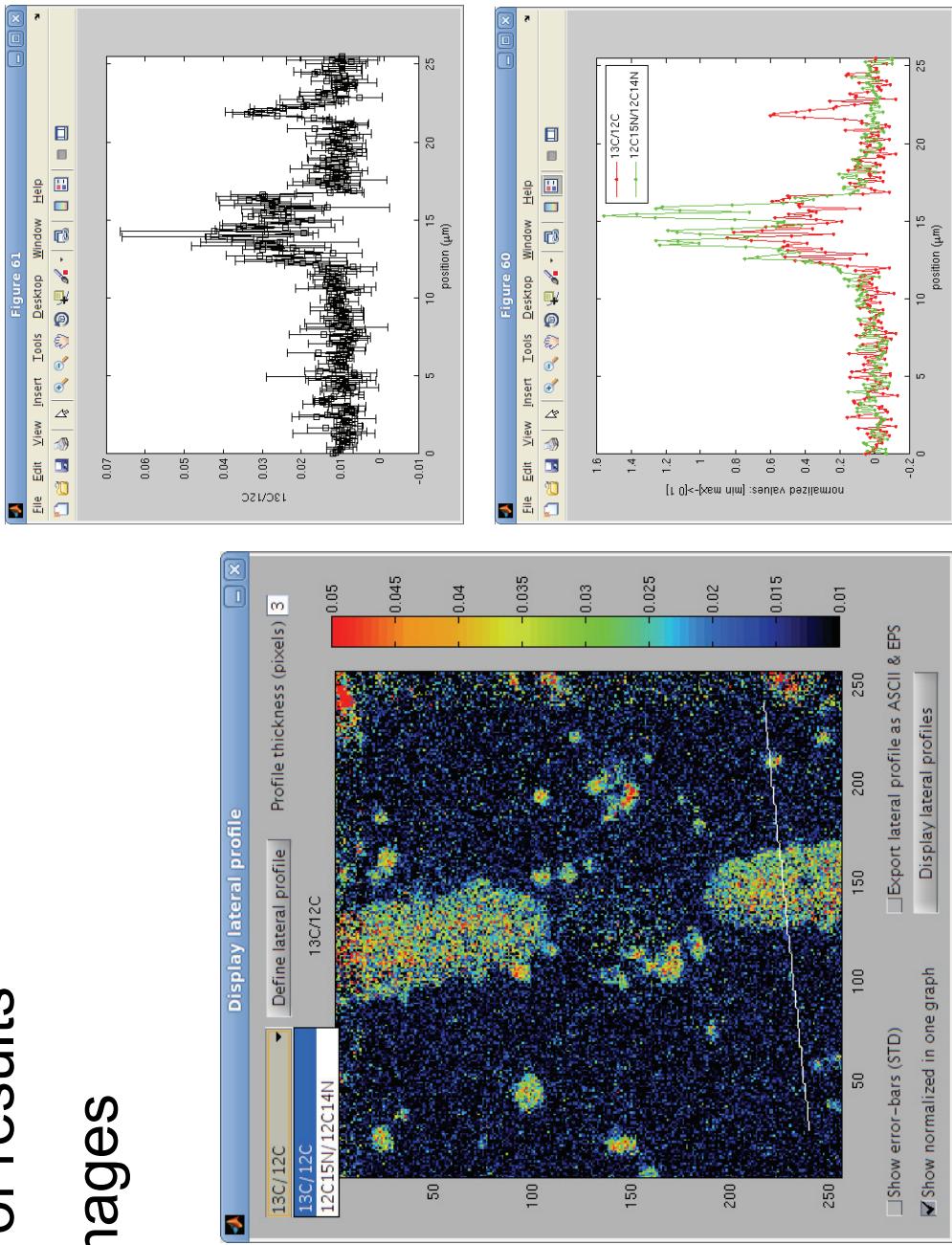
Common features

- Display and export of results
- Mass & Ratio images
- RGB overlays



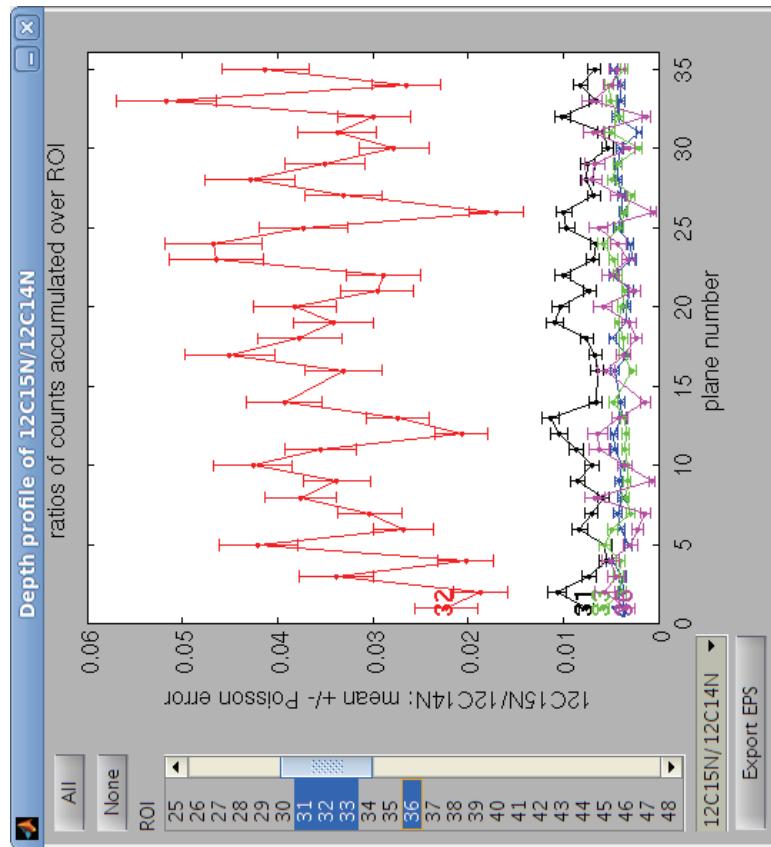
Common features

- Display and export of results
- Mass & Ratio images
- RGB overlays
- Lateral profiles



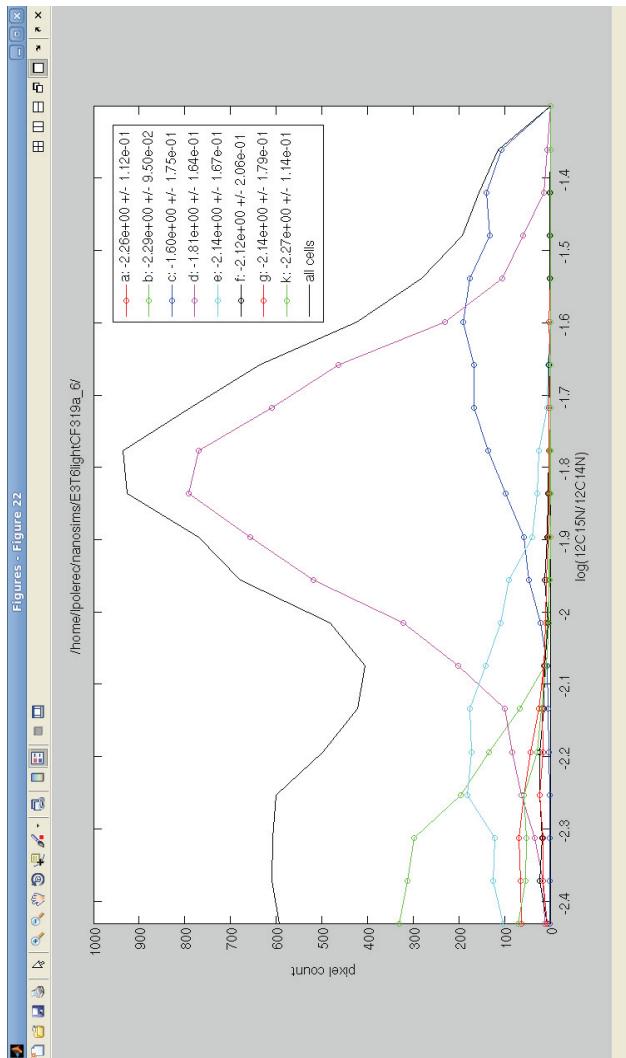
Common features

- Display and export of results
- Mass & Ratio images
- RGB overlays
- Lateral profiles
- Depth profiles



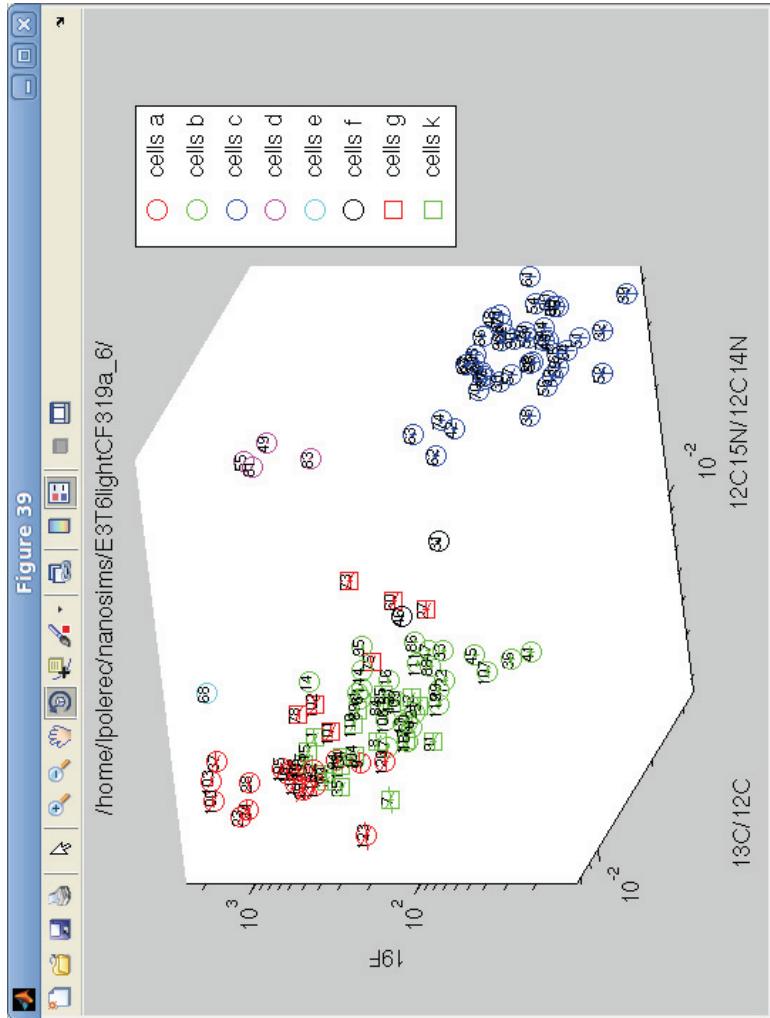
Common features

- Display and export of results
- Mass & Ratio images
- RGB overlays
- Lateral profiles
- Depth profiles
- Histograms
 - All ROIs
 - Separate for ROI classes



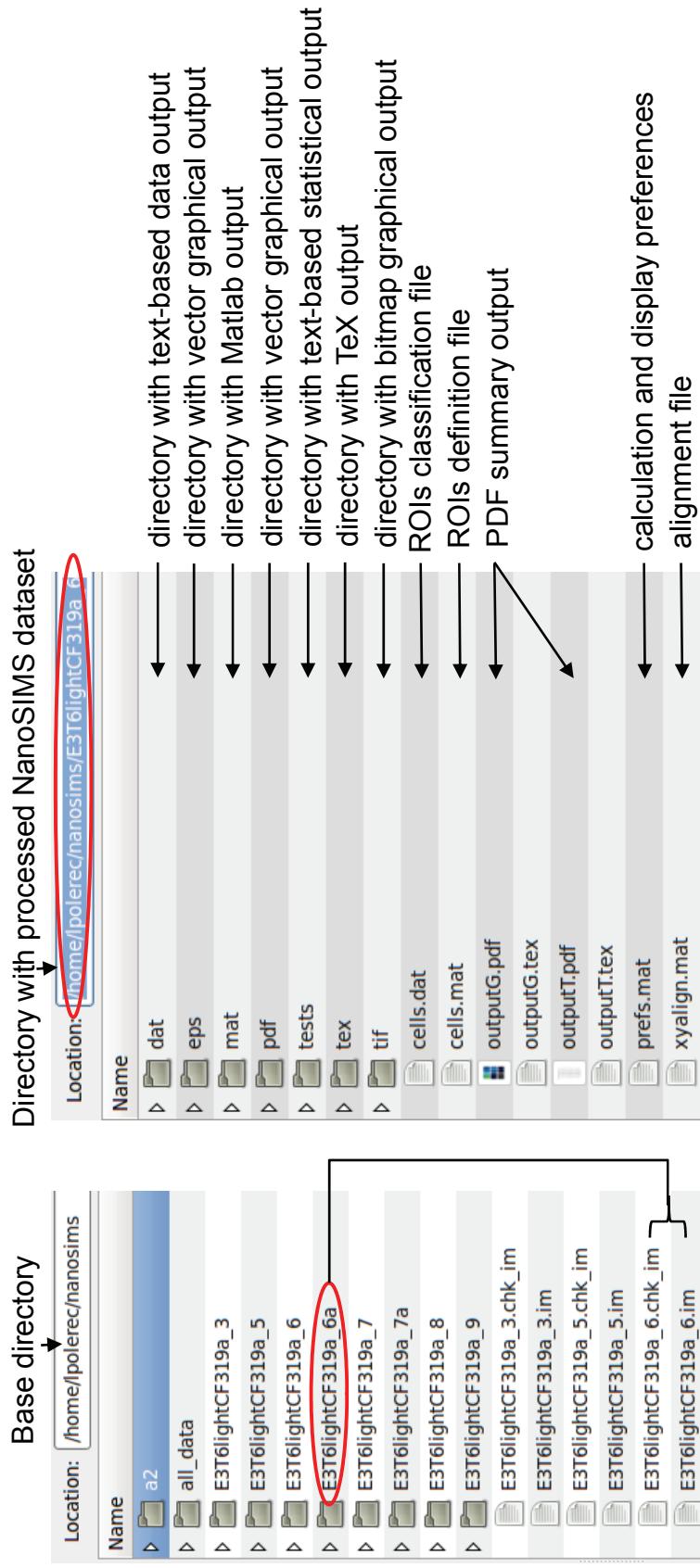
Common features

- Display and export of results
- Mass & Ratio images
- RGB overlays
- Lateral profiles
- Depth profiles
- Histograms
- Scatter plots
 - All ROIs
 - Separate for ROI classes



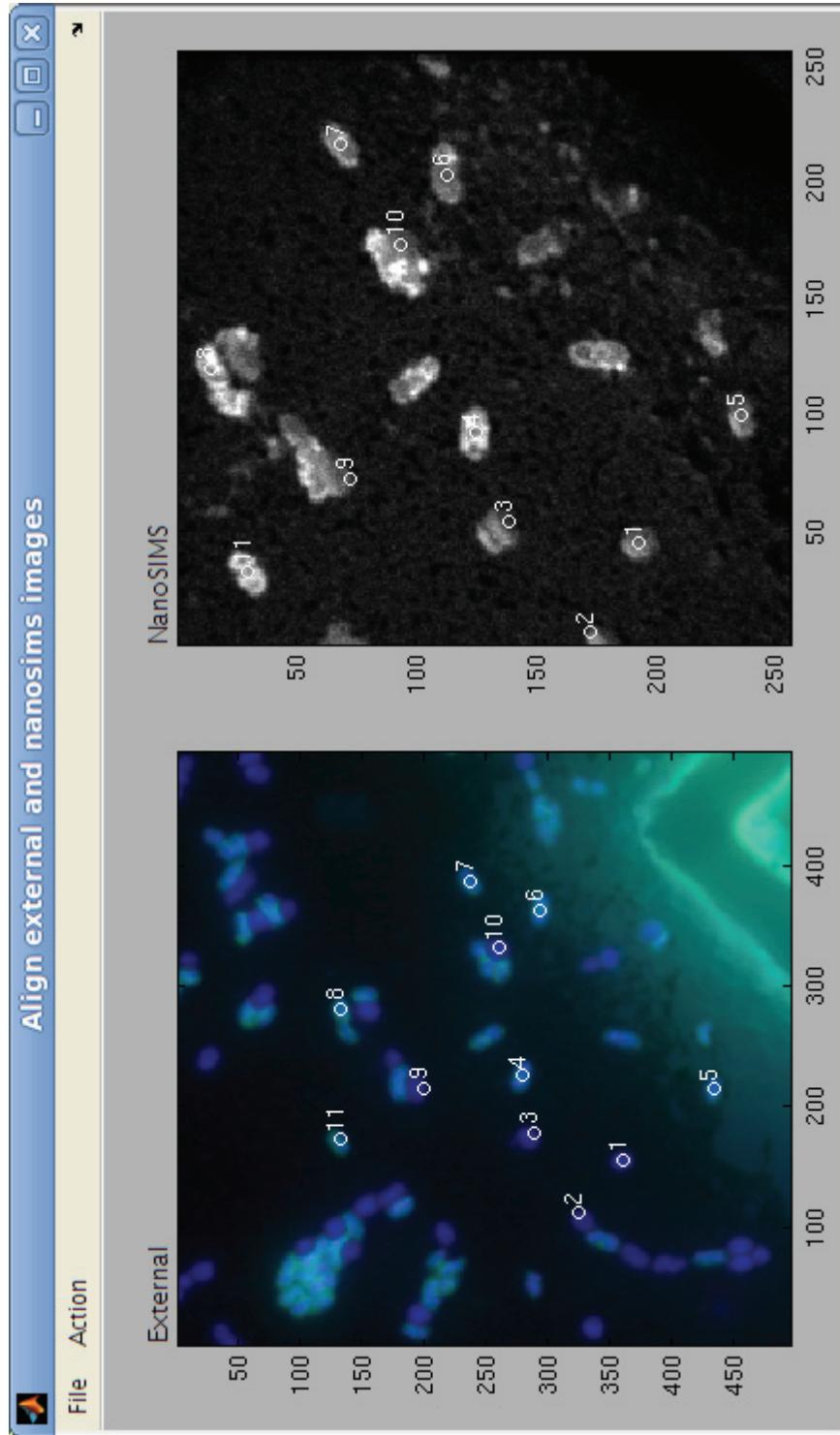
Common features

- Display and export of results
 - Graphical
 - ASCII text



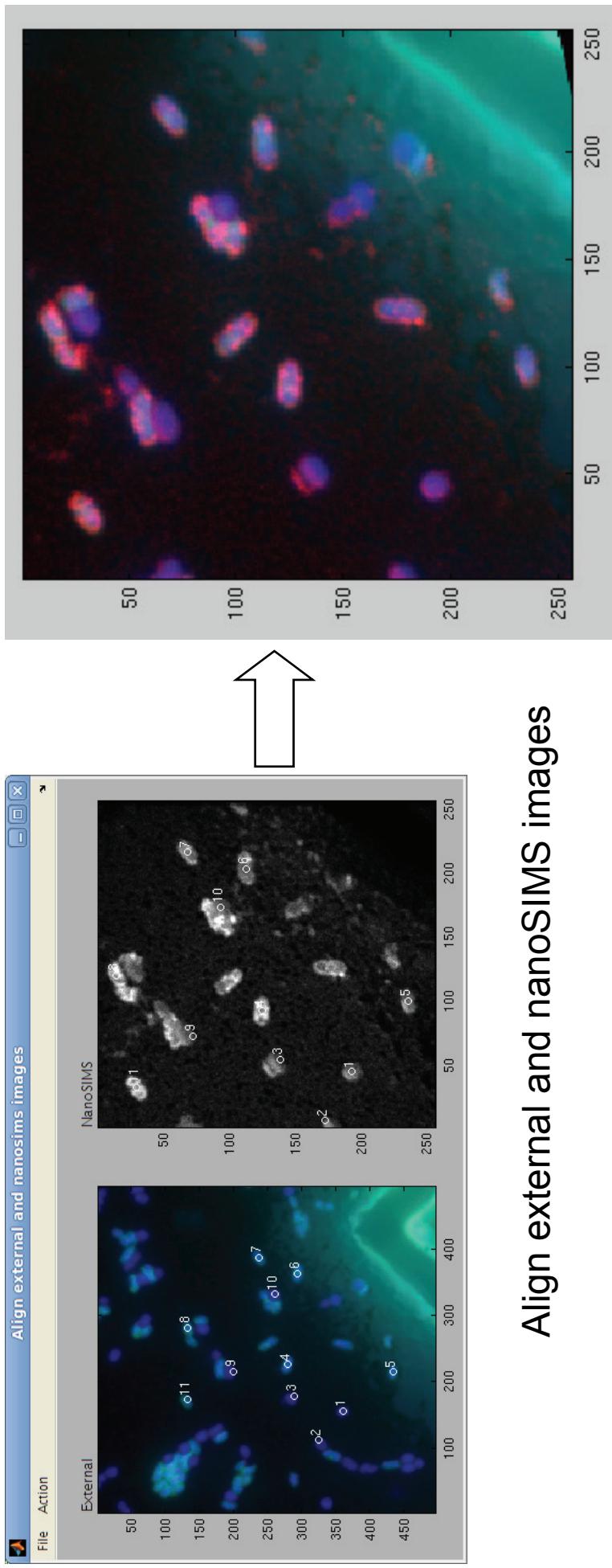
Novel features

- ROI definition based on an external image (e.g. FISH, SEM, AFM)



Novel features

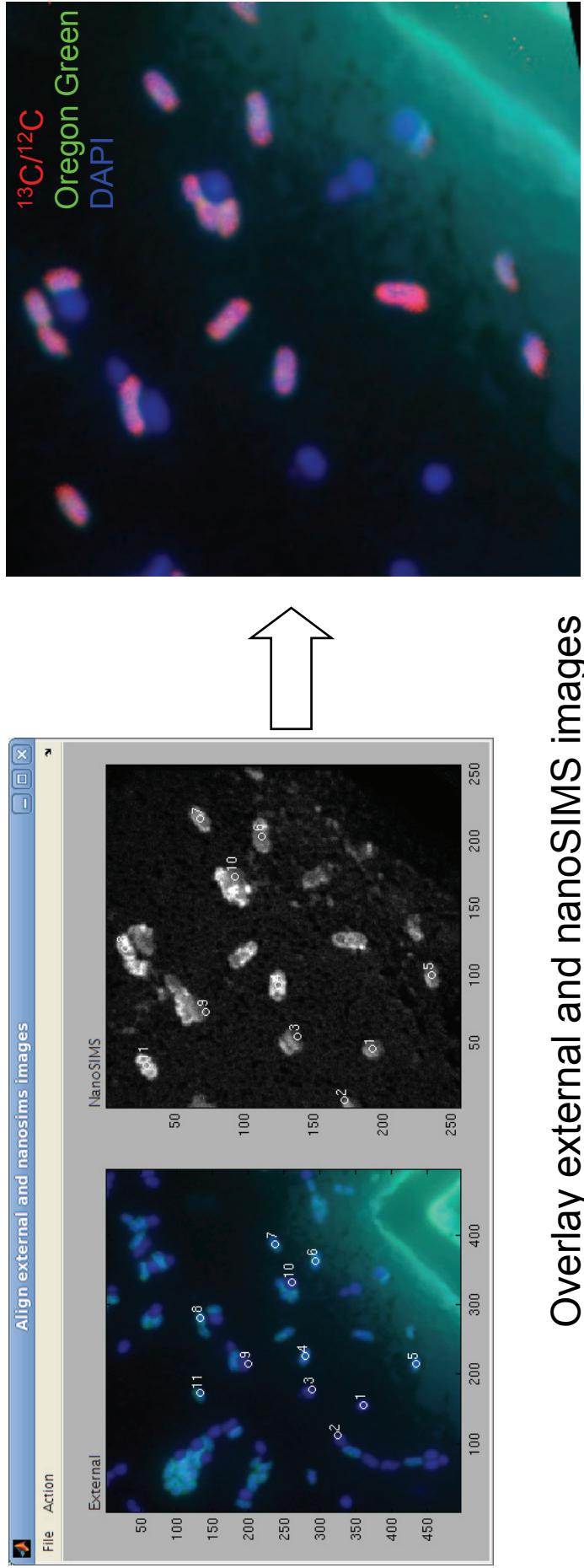
- ROI definition based on an external image (e.g. FISH, SEM, AFM)



Align external and nanosIMS images

Novel features

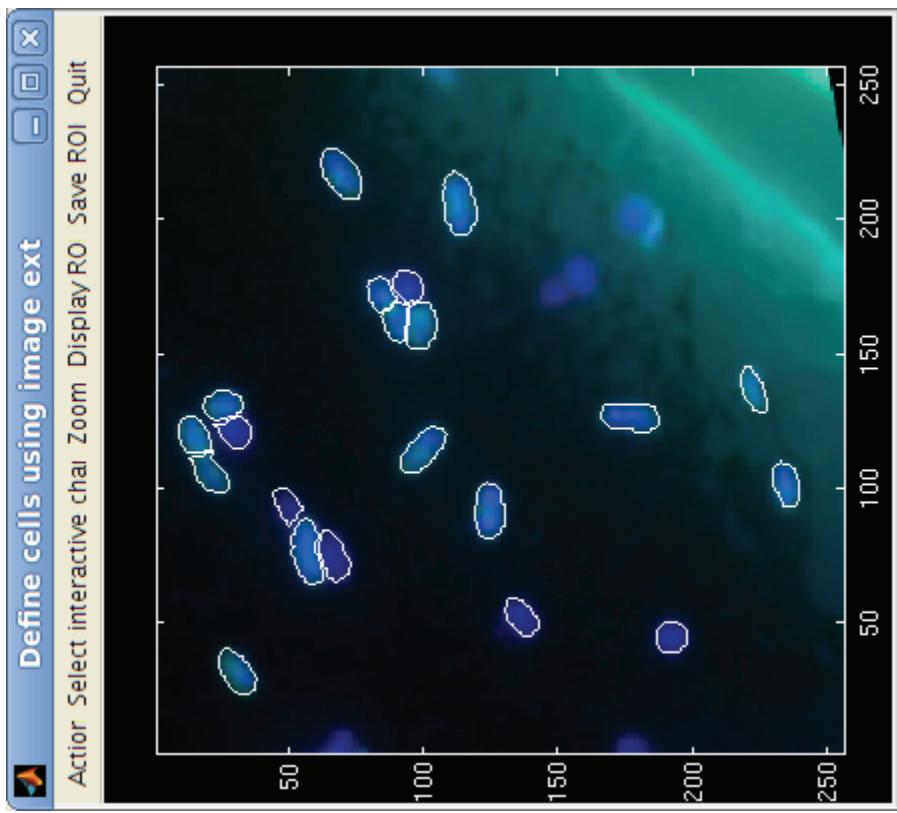
- ROI definition based on an external image (e.g. FISH, SEM, AFM)



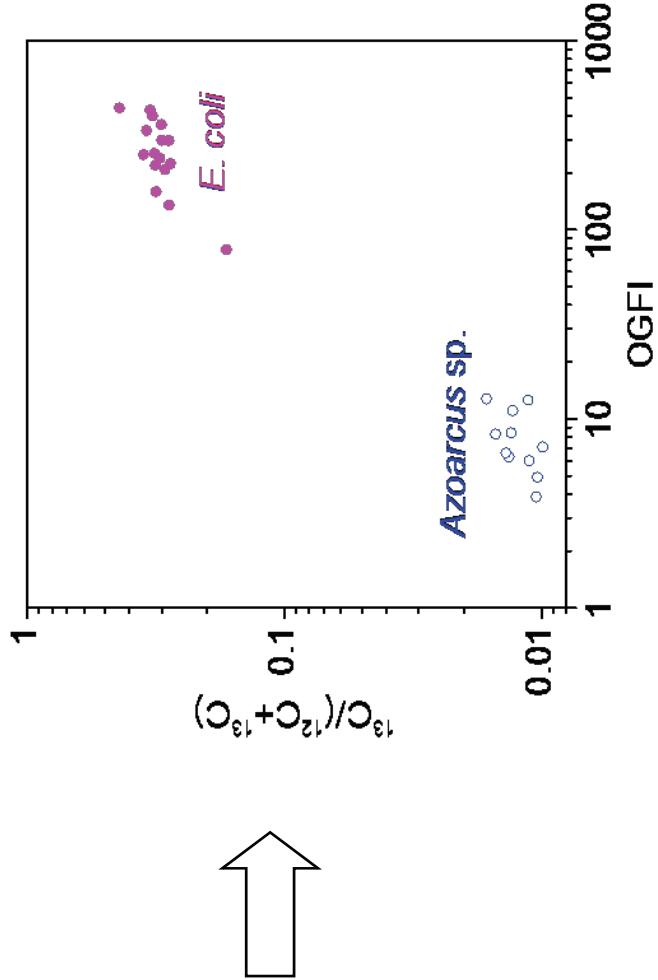
Overlay external and nanoSIMS images

Novel features

- ROI definition based on an external image (e.g. FISH, SEM, AFM)



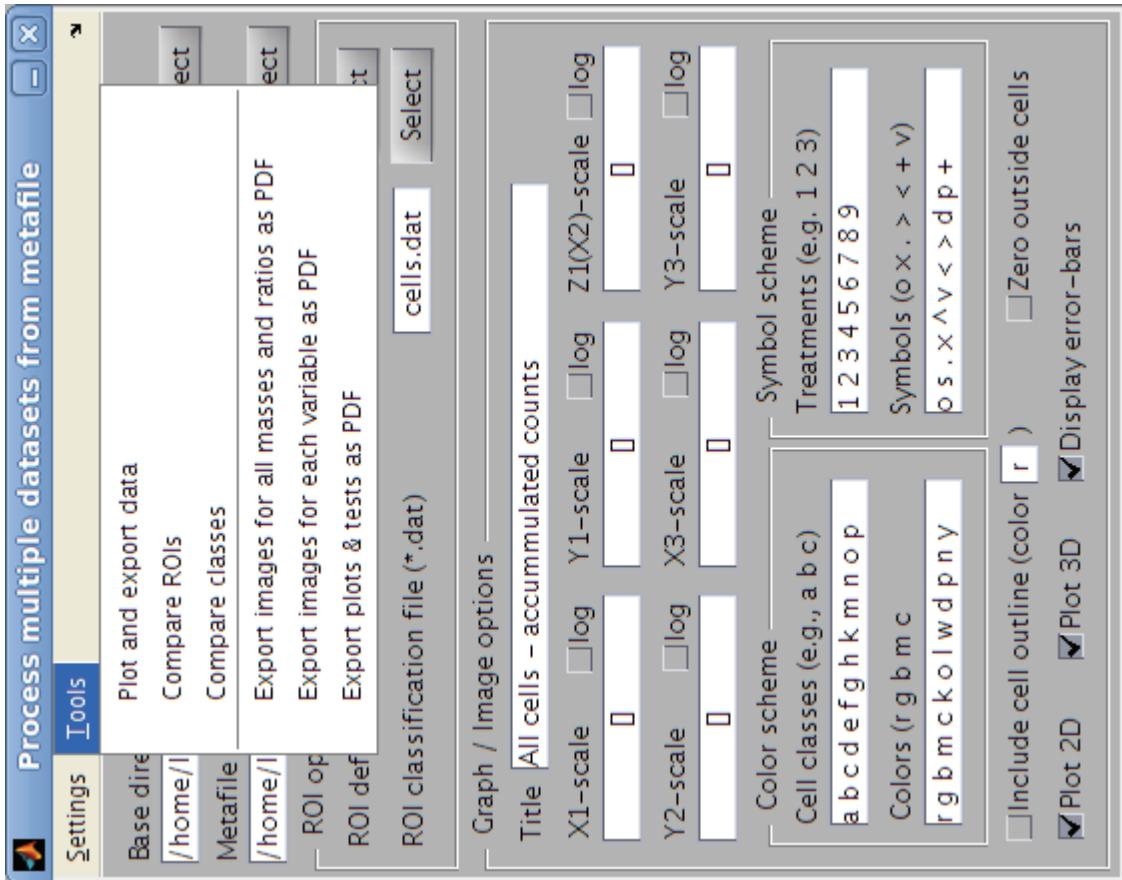
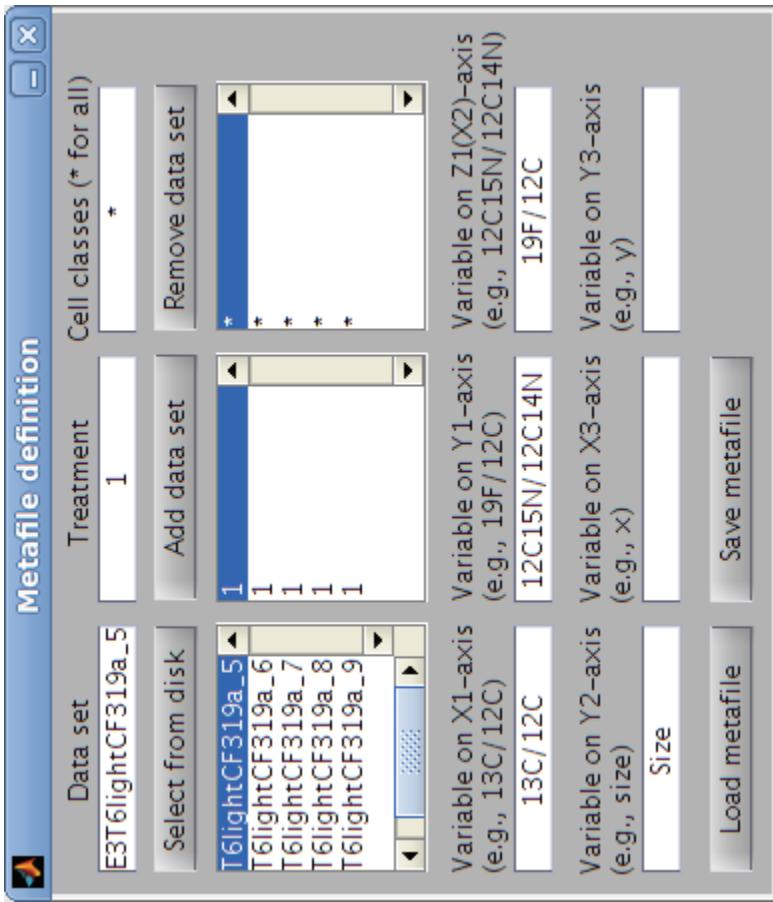
Use external image to define ROIs



Plot FISH intensities (identity) versus nanoSIMS results ($^{13}\text{C}/^{12}\text{C}$ - activity)

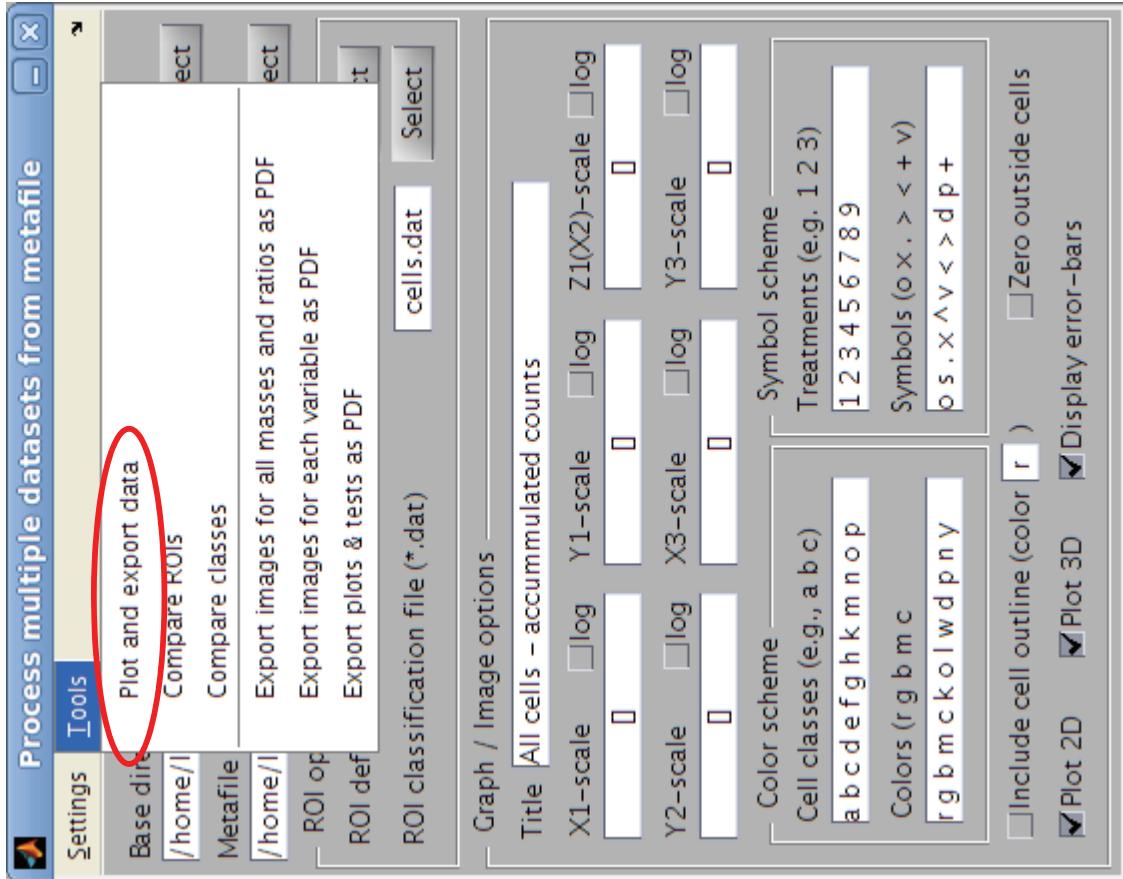
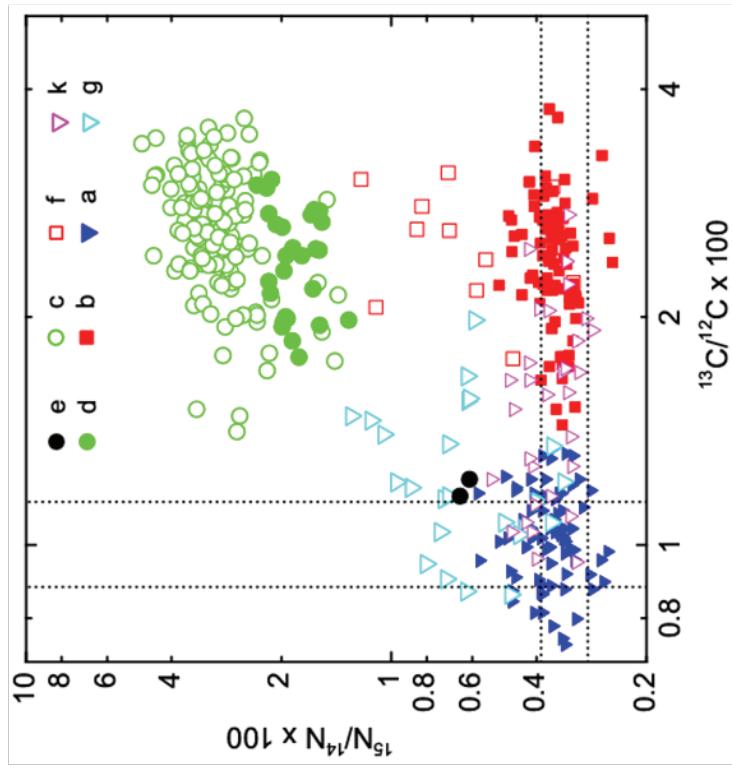
Novel features

- Metafile processing
 - = Combine results from multiple datasets into one



Novel features

- Metafile processing



Complication of results from 5 datasets
- scatter plot for ~480 ROIs

Novel features

- Metafile processing

The screenshot shows the software interface for processing multiple datasets from a metafile. The main window displays 12 processed images arranged in a grid. Each image shows a heatmap of cell distribution with various color overlays and boundary outlines. Below the images is a configuration panel with several tabs and sections:

- Settings** tab:
 - Base directory: /home/l
 - Metafile: /home/l
 - ROI definition file: ROI.def
- Tools** tab:
 - Plot and export data
 - Compare ROIs
 - Compare classes
 - Export images for all masses and ratios as PDF** (highlighted with a red oval)
 - Export images for each variable as PDF** (highlighted with a red oval)
 - Export plots & tests as PDF**
- ROI classification file (*.dat)**: cells.dat
- Select** button
- Graph / Image options** section:
 - Title: All cells – accumulated counts
 - X1-scale: log (unchecked)
 - Y1-scale: log (unchecked)
 - Z1(X2)-scale: log (unchecked)
 - X2-scale: log (unchecked)
 - Y2-scale: log (unchecked)
 - X3-scale: log (unchecked)
 - Y3-scale: log (unchecked)
 - Z2-scale: log (unchecked)
- Color scheme** section:
 - Cell classes (e.g., a b c): a b c d e f g h k m n o p
 - Colors (r g b m c): r g b m c k o l w d p n y
- Symbol scheme** section:
 - Treatments (e.g. 1 2 3): 1 2 3 4 5 6 7 8 9
 - Symbols (o x . > < + v): o s . x ^ v < > d p +
- Plot options** section:
 - Include cell outline (color r) (unchecked)
 - Zero outside cells (unchecked)
 - Plot 3D (checked)
 - Display error-bars (checked)

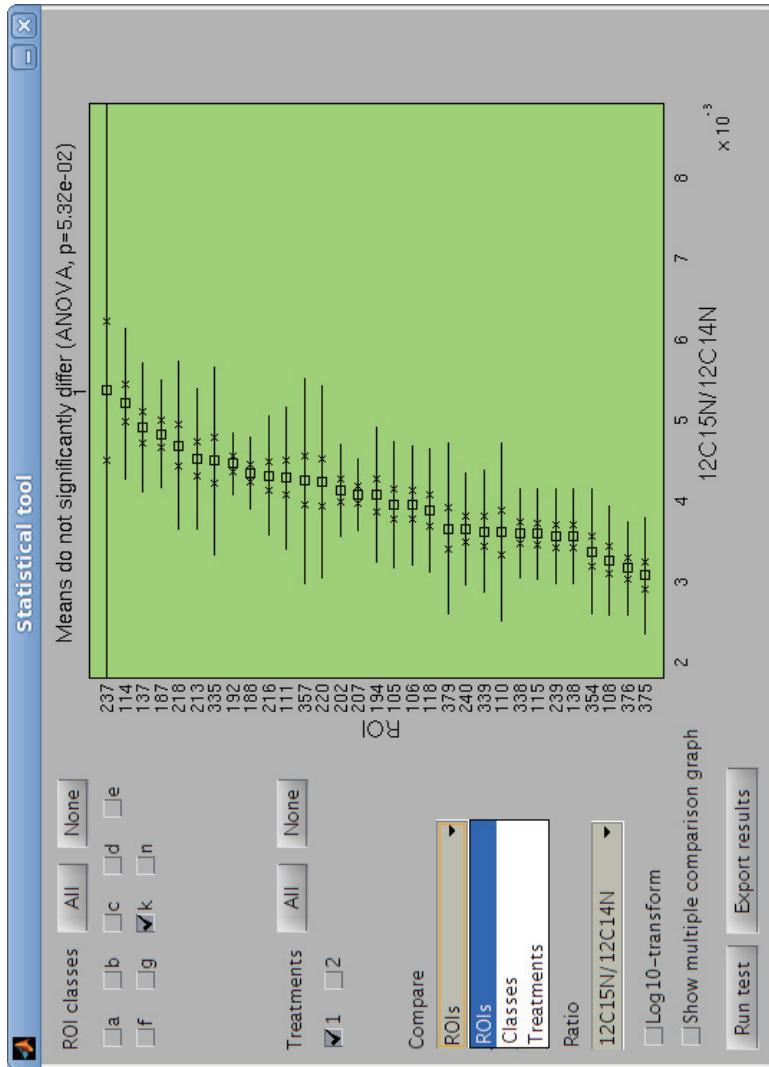
Complication of results from 12 datasets
- images

Novel features

- Statistical comparison

Novel features

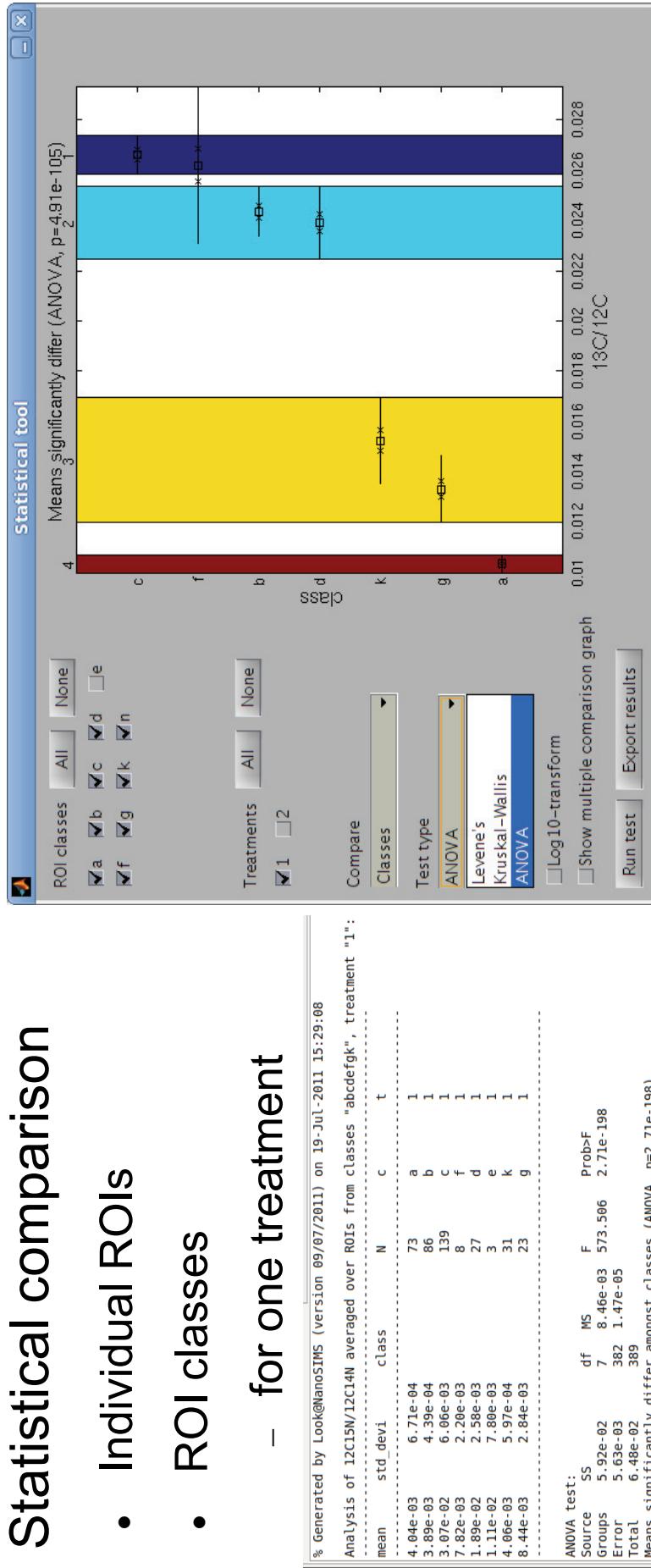
- Statistical comparison
 - Individual ROIs
 - for one class and one treatment



Based on depth-profiles = replicate measurements

Novel features

- Statistical comparison
 - Individual ROIs
 - ROI classes
 - for one treatment

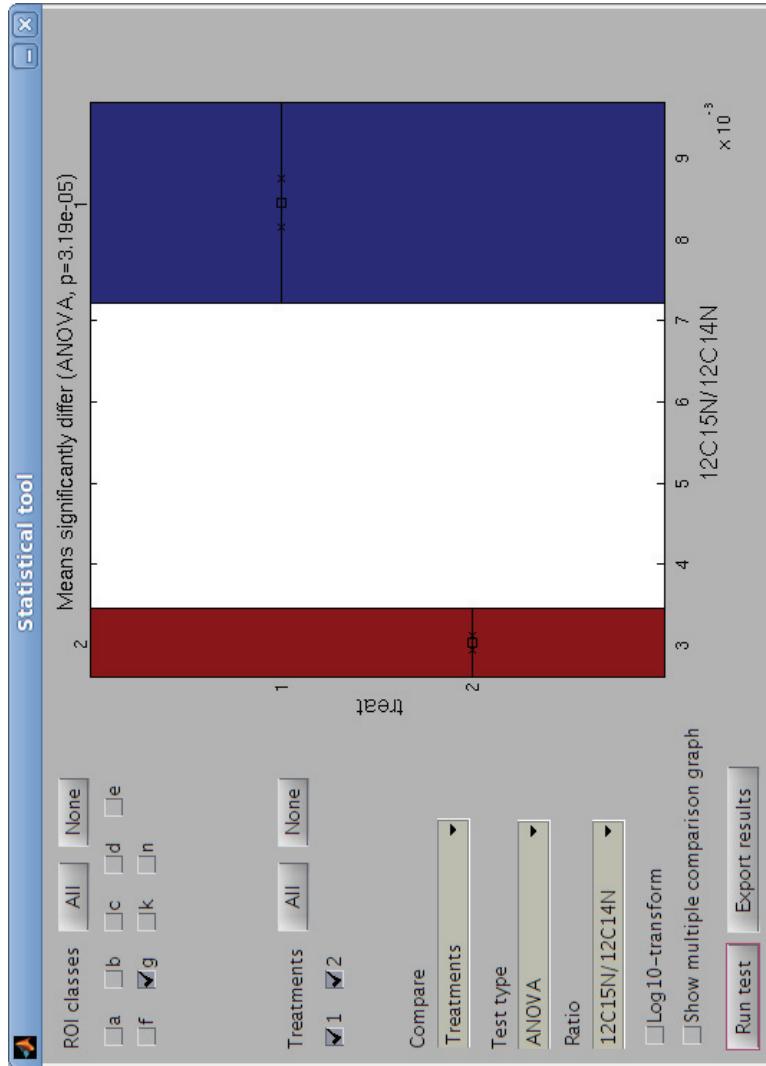


Graphical and text output

Ranking based on multiple comparison of the means:					
	class	MEAN	MEAN (95%)	SEM	[Ruin Max]
C	0.03076	(0.02968 0.03172)	0.00051	[1 1]	
d	0.01885	(0.01783 0.01987)	0.00050	[2 2]	
e	0.01109	(-0.00829 0.03848)	0.00450	[3 4]	
g	0.00844	(0.00721 0.00967)	0.00059	[3 3]	
f	0.00782	(0.00598 0.00966)	0.00078	[3 4]	
a	0.00486	(0.00384 0.00428)	0.00011	[4 4]	
k	0.00404	(0.00388 0.00426)	0.00008	[4 4]	
b	0.00389	(0.00380 0.00399)	0.00005	[4 4]	

Novel features

- Statistical comparison
 - Individual ROIs
 - ROI classes
 - Treatments
 - for one class



Summary

- Look@NanoSIMS

- Free, open-source (Matlab, platform-independent)
- Custom-made for environmental microbiologists but useful for scientists in any other discipline
- Common features
 - Drift-corrected alignment, interactive ROI definition, ROI classification, display and export of results (images, RGB overlays, histograms, scatter plots, depth and lateral profiles)
- Novel features
 - ROI definition based on an external image
 - Statistical comparison of ROIs, ROI classes, treatments
 - Rapid compilation of results from multiple datasets

How to use the program: details in....

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Nanometre-scale secondary ion mass spectrometry (nanoSIMS) allows quantitative analysis of elemental and isotopic composition of a sample with a submicrometre spatial resolution, and has been applied in diverse

nanoSIMS fields ranging from ancient and modern marine



Steps of the analysis

- Install the program
 - Matlab, Look@Nanosims, TeX, ghostscript
- Organize your data
- Analyze separately each scan from your replicate measurements
 - Accumulate planes, define ROIs
 - Perform quality checks: depth profiles, lateral profiles, statistics
 - Calculate ratios, export results as text or images
- Combine results from these analyses
 - Plot, statistically analyze
- Interpret results based on the statistical analyses

Things to remember

- Save analysis preferences for each dataset for future reference
 - Report errors or bugs by email; submit screenshot of the error together with the raw data and a brief description what you have done before the error occurred.
- Read and follow messages in the Matlab's command window
 - Share your ideas for other types of analyses. There is a good chance that they will be relatively quickly implemented in the program.
- Define ROIs slowly and carefully; you won't have to define them again after a critical assessment of your supervisor/collaborator
 - Newest version will be available via www.microsen-wiki.net or via Dropbox (send me an email and I will share the folder with you).
- 12C14N or 32S are usually good indicators for biomass
 - Nanosims scans can be distorted relative to images obtained by other techniques (e.g. FISH); define enough reference points for satisfactory alignment
 - When defining ROIs based on external images or signals other than 12C14N or 32S (e.g. ratios), always perform cross-checking against 12C14N or 32S, if possible.
- Acknowledge the developer team. The program didn't fall from the sky.
- When defining a ROI, always finish the started operation cleanly, or press 'Esc' to quit the operation; for example, never select ROI definition by interactive thresholding and then immediately select another type of ROI definition without actually finishing the definition by interactive thresholding!
- Save the defined ROIs occasionally, or more frequently. There is no guarantee that, because of the improper handling of the ROI definition procedure described in the previous point, the ROI definition tool will not crash, leaving you with a lot of careful work done in vain.