Figure S1: Images of secondary ions ($^{12}C^-$, $^{13}C^-$, $^{19}F^-$), ion pairs ($^{12}C^{14}N^-$, $^{12}C^{15}N^-$) and secondary electrons (SE) detected for the environmental sample from the Baltic Sea. Gray-bar represents total ion counts accumulated over 35 planes (dwelling time per plane: 1 ms).
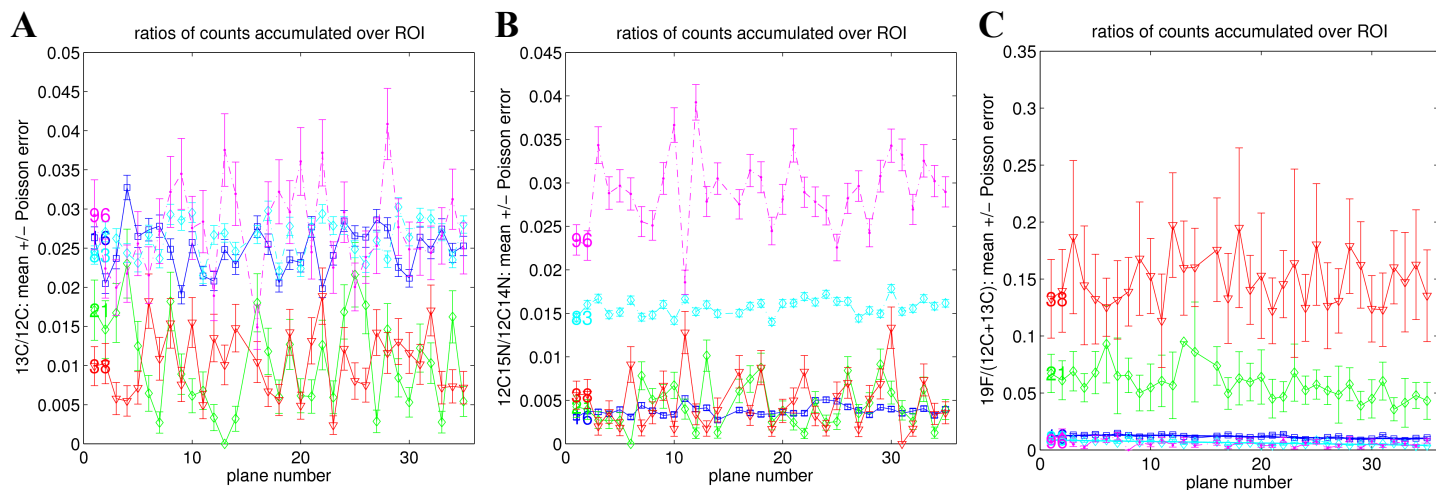
Figure S2: (A-C) Depth profiles of ratios $^{13}C/^{12}C$, $^{15}N/^{14}N$ and $^{19}F/(^{12}C+^{13}C)$ in selected ROIs, derived from ion counts accumulated over ROI pixels in each plane. Symbols represent mean ratio values, error-bars correspond to Poisson errors (see Eqs. 3 and 5 in *Experimental procedures*).
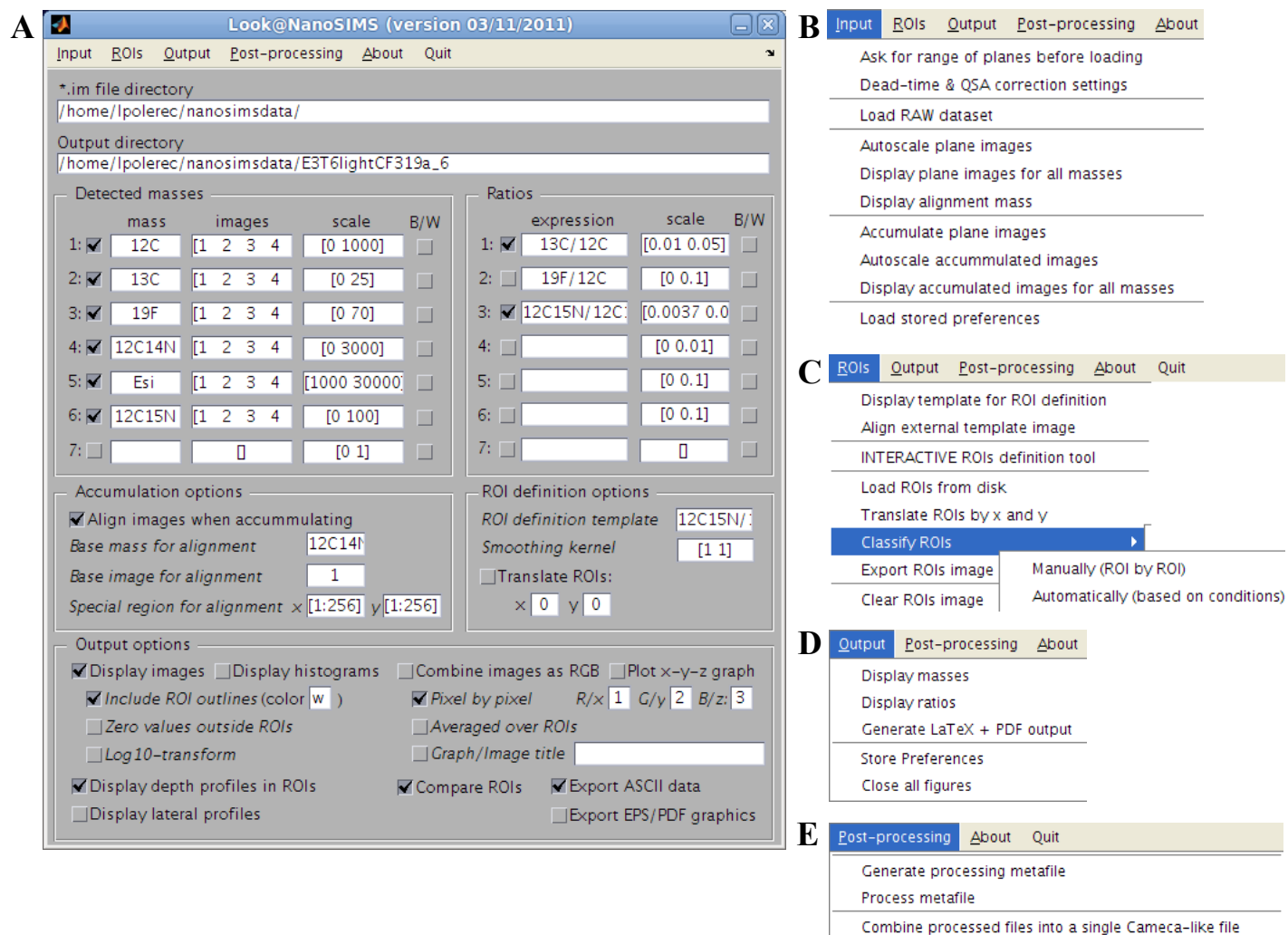
Figure S3: (**A**) Main graphical user interface (GUI) of the Look@NanoSIMS program. (**B**) *Input* menu is used to define dead-time and QSA correction settings, load nanoSIMS datasets from disk, and to visually inspect, align and accumulate plane images. Alignment and plane accumulation are controlled by the parameters in the *Accumulation options* box and by *images* in the *Detected masses* box. (**C**) *ROIs* menu is used to interactively define regions of interest (ROIs), to classify ROIs, to export ROI outlines in a graphical output, and to interactively align an external image with nanoSIMS images if it was chosen as template for ROI definition. The ROI definition is controlled by the parameters in the *ROIs definition options* box. (**D**) *Output* menu is used to process and export data for the currently processed dataset. The type of data processing performed is controlled by the parameters in the *Output options* box, allowing display and export of detected masses and calculated mass ratios as values accumulated over ROIs, images, histograms, depth profiles, lateral profiles, composite RGB images and scatter plots. This menu also allows statistical comparison of ROIs and ROI classes. (**E**) *Post-processing* menu is used to process results obtained by the analysis of multiple datasets.

A base directory

Location:	/home/lpolerec/nanosims

Name

▷ 📁 a2
▷ 📁 all_data
▷ 📁 E3T6lightCF319a_3
▷ 📁 E3T6lightCF319a_5
▷ 📁 E3T6lightCF319a_6
▷ 📁 E3T6lightCF319a_6a
▷ 📁 E3T6lightCF319a_7
▷ 📁 E3T6lightCF319a_7a
▷ 📁 E3T6lightCF319a_8
▷ 📁 E3T6lightCF319a_9
📄 E3T6lightCF319a_3.chk_im
📄 E3T6lightCF319a_3.im
📄 E3T6lightCF319a_5.chk_im
📄 E3T6lightCF319a_5.im
📄 E3T6lightCF319a_6.chk_im
📄 E3T6lightCF319a_6.im

B Directory with processed NanoSIMS dataset

Location: /home/lpolerec/nanosims/E3T6lightCF319a_6

Name

▷ 📁 dat ← directory with text-based data output
▷ 📁 eps ← directory with vector graphical output
▷ 📁 mat ← directory with matlab output
▷ 📁 pdf ← directory with vector graphical output
▷ 📁 tests ← directory with text-based statistical output
▷ 📁 tex ← directory with tex output
▷ 📁 tif ← directory with bitmap graphical output
📄 cells.dat ← ROIs classification file
📄 cells.mat ← ROIs definition file
📄 outputG.pdf ← PDF summary output
📄 outputG.tex
📄 outputT.pdf
📄 outputT.tex
📄 prefs.mat ← calculation and display preferences
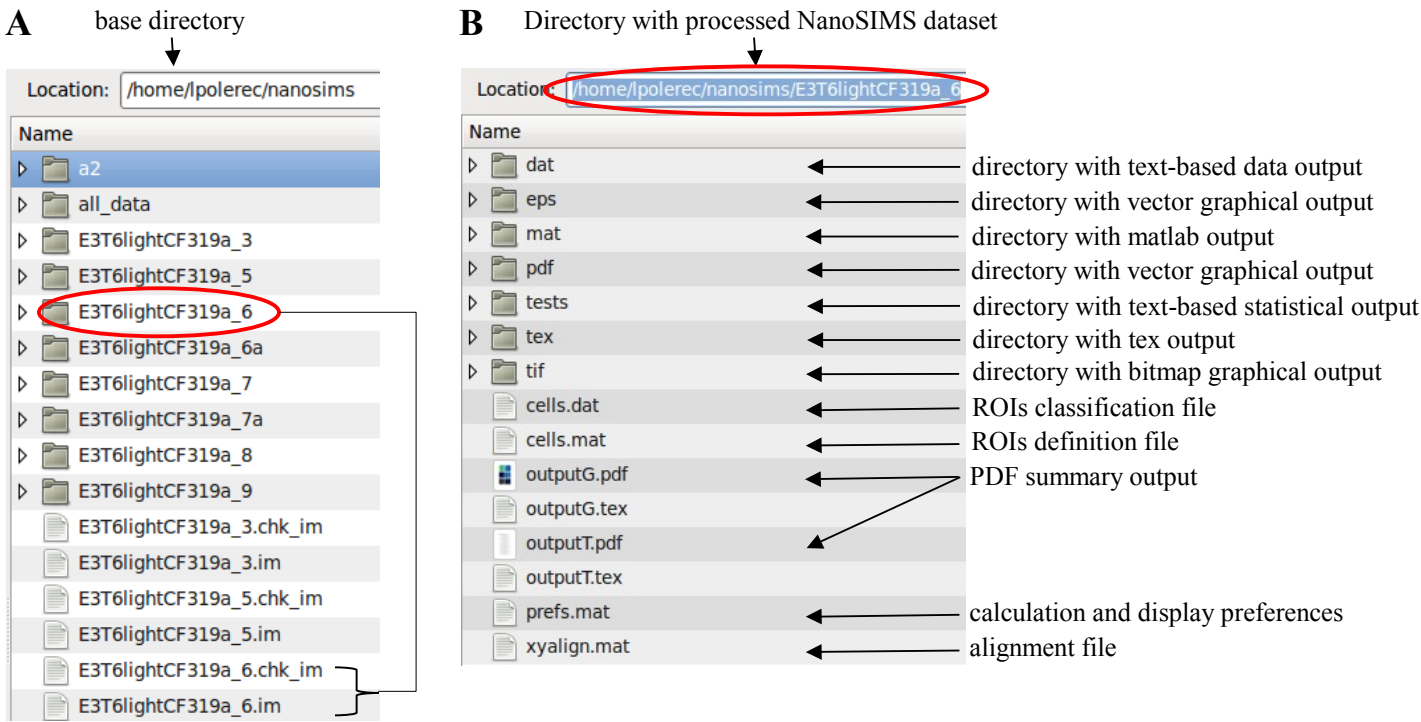📄 xyalign.mat ← alignment file

Figure S4: Organization of the data generated by the Look@NanoSIMS program. Datasets that belong to a specific experiment and are intended for later synthesis by metafile processing must be copied in the same base directory (panel **A**). For each nanoSIMS dataset (files with extension **im** and **chk_im**), a directory with the same base name as the dataset is automatically created during the analysis. This directory contains all information generated during the data analysis (panel **B**). File **prefs.mat** contains settings of the analysis, and must be saved manually by selecting *Store Preferences* in the *Output* menu after the analysis of each dataset.
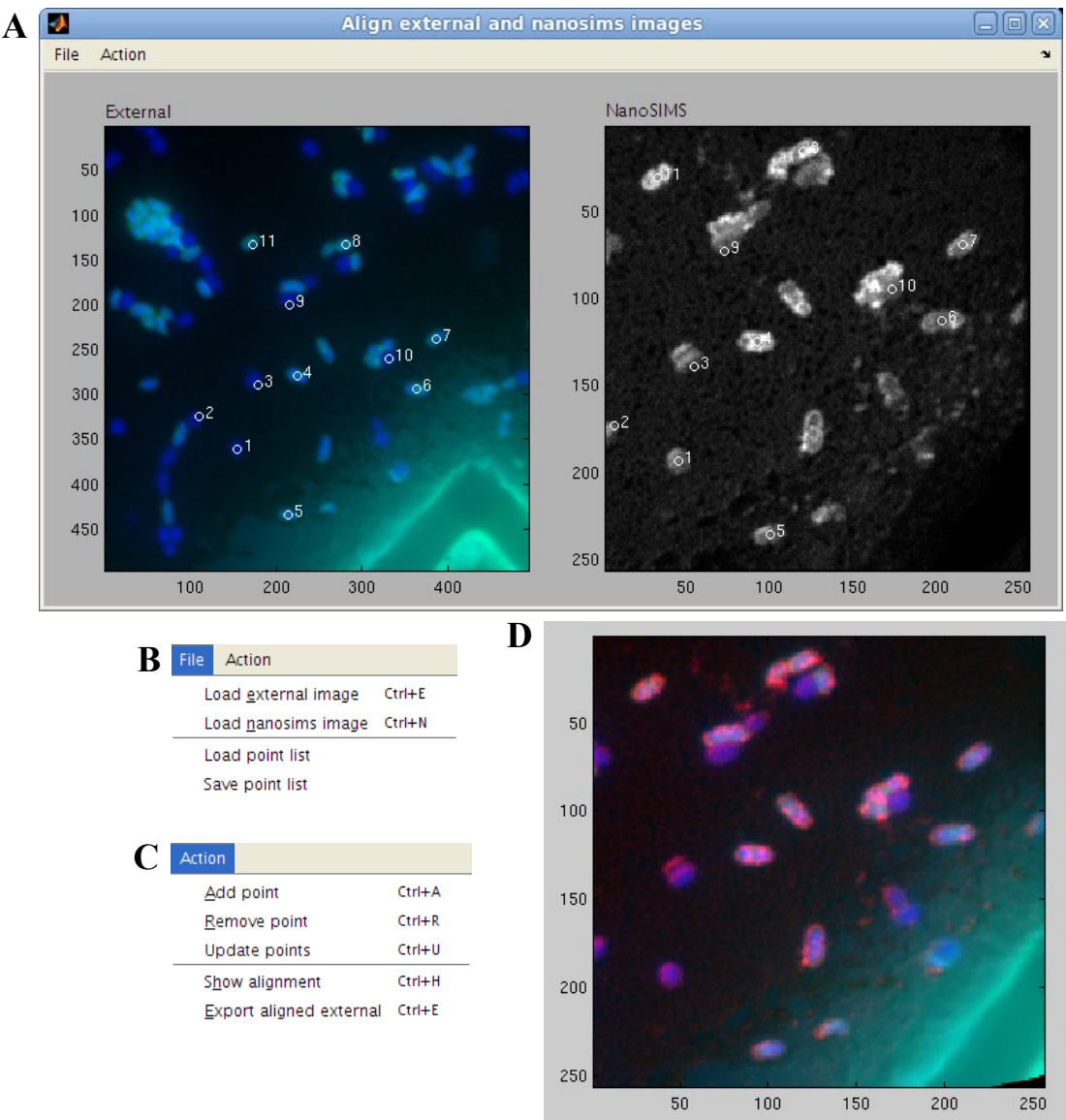
Figure S5: (**A**) Graphical user interface for interactive alignment of the external and nanoSIMS ion images. Images are loaded through the *File* menu (**B**). Reference points that correspond to each other are defined interactively in parallel for both images through the *Action* menu (**C**). At least 5 reference points are usually needed to account for unequal magnification of, or correct for distortion between the two images to achieve satisfactory alignment. After visual verification of the alignment (**D**), the aligned external image as well as the reference points are saved on disk through the *Action* and *File* menu, respectively.
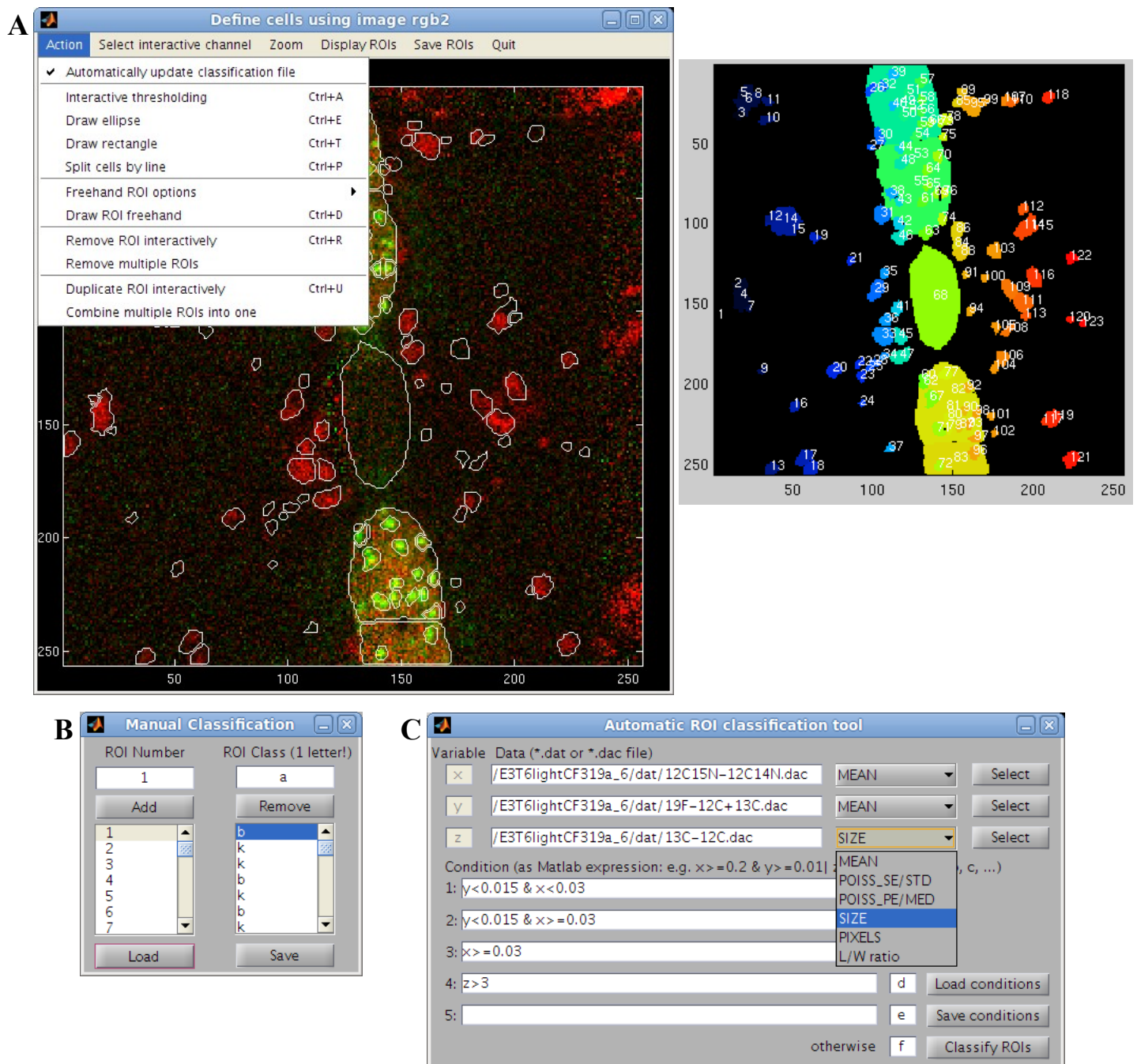
Figure S6: Graphical user interface for (**A**) interactive ROI definition, (**B**) manual and (**C**) automatic ROI classification. ROI definition is controlled through the *Action* menu, allowing interactive addition and removal of ROIs. When ROIs are well separated, *interactive thresholding* is the preferred method for ROI definition due to its speed and reproducibility. When a composite RGB image is used as template for ROI definition, the active channel used in the interactive thresholding mode is selected through the *Select interactive channel* menu. The currently defined ROIs and their identification numbers (right panel in **A**) are displayed by selecting *Display ROIs* from the menu. Manual ROI classification (**B**) is done by manually assigning a class name to each ROI identification number. The class name should consist of one alphabetical character. Automatic ROI classification (**C**) is based on logical conditions that can be specified for up to 3 variables. The variable values are taken from the files and specific columns defined in the *Data* fields, the conditions are specified in the *Condition* fields using Matlab syntax.
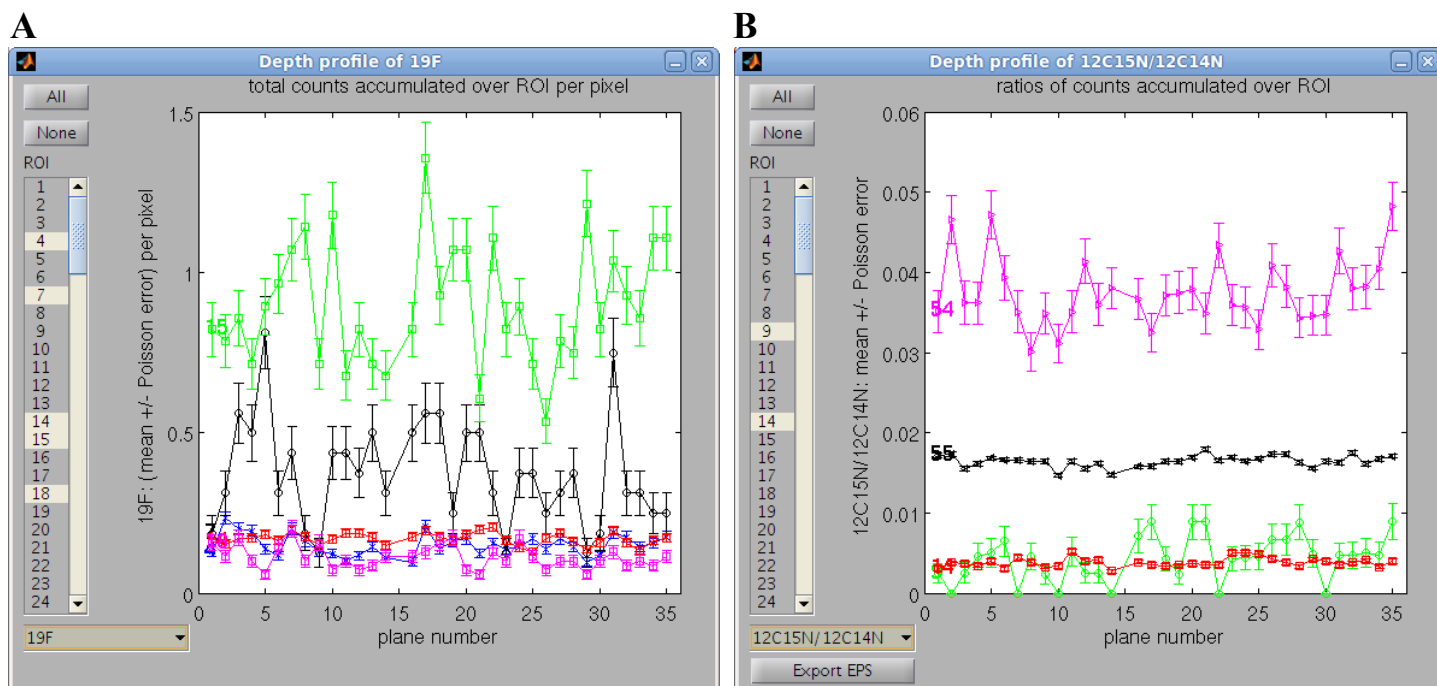
Figure S7: Graphical user interface for interactive display of depth profiles of (**A**) masses and (**B**) ratios in defined ROIs. Symbols in (**A**) correspond to total ion counts accumulated over ROI pixels in a given plane per dwelling time, and are normalized to the amount of pixels in the ROI. Symbols in (**B**) correspond to ratios of total ion counts accumulated over ROI pixels in a given plane per dwelling time. Length of the error bars represents the corresponding Poisson errors (see Eqs. 3 and 5 in *Experimental procedures*). The type of mass and mass ratio displayed is specified through the pull-down menu, ROIs for which depth profiles should be displayed are selected in the listbox. Due to limitations of the graphical user interface, maximum of 50 ROIs can be selected at once.
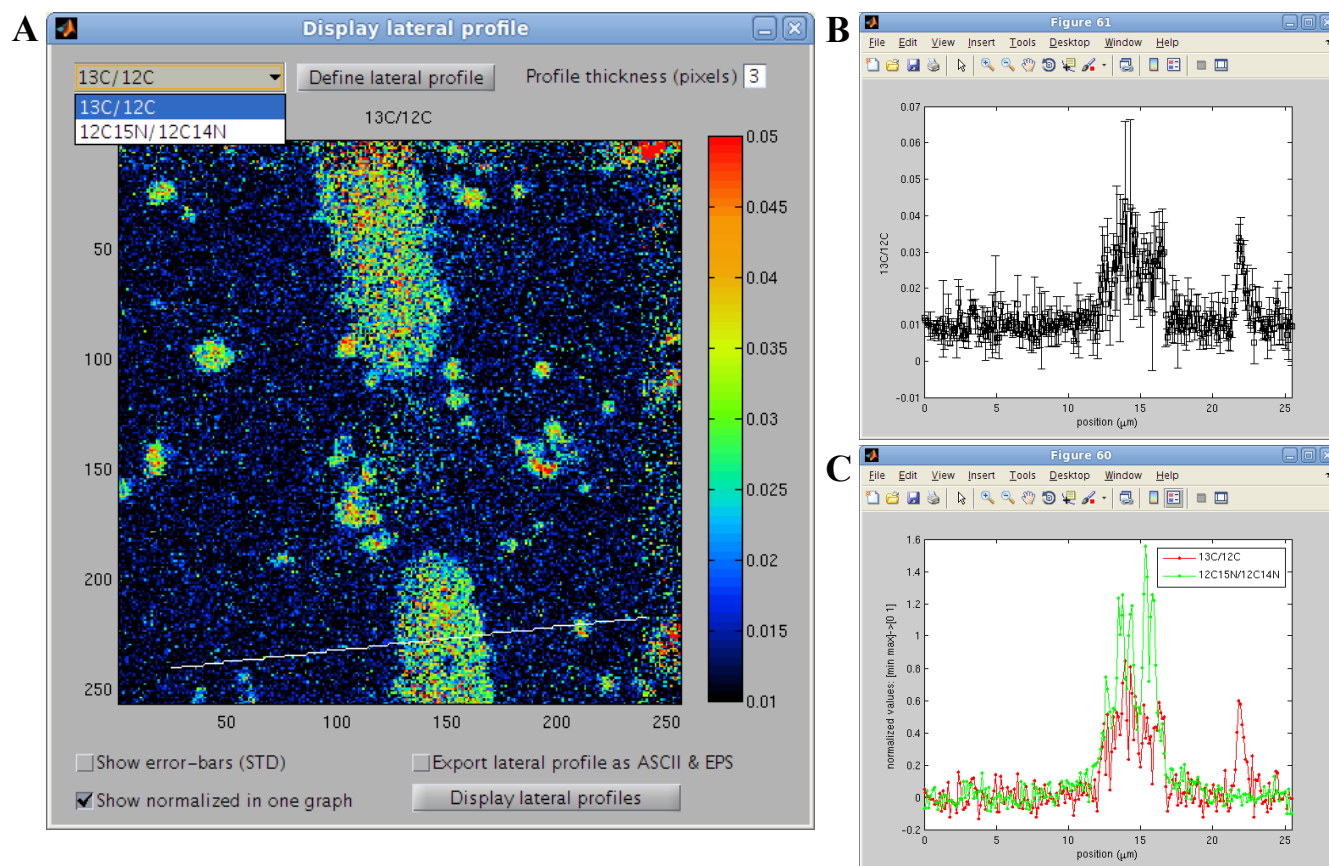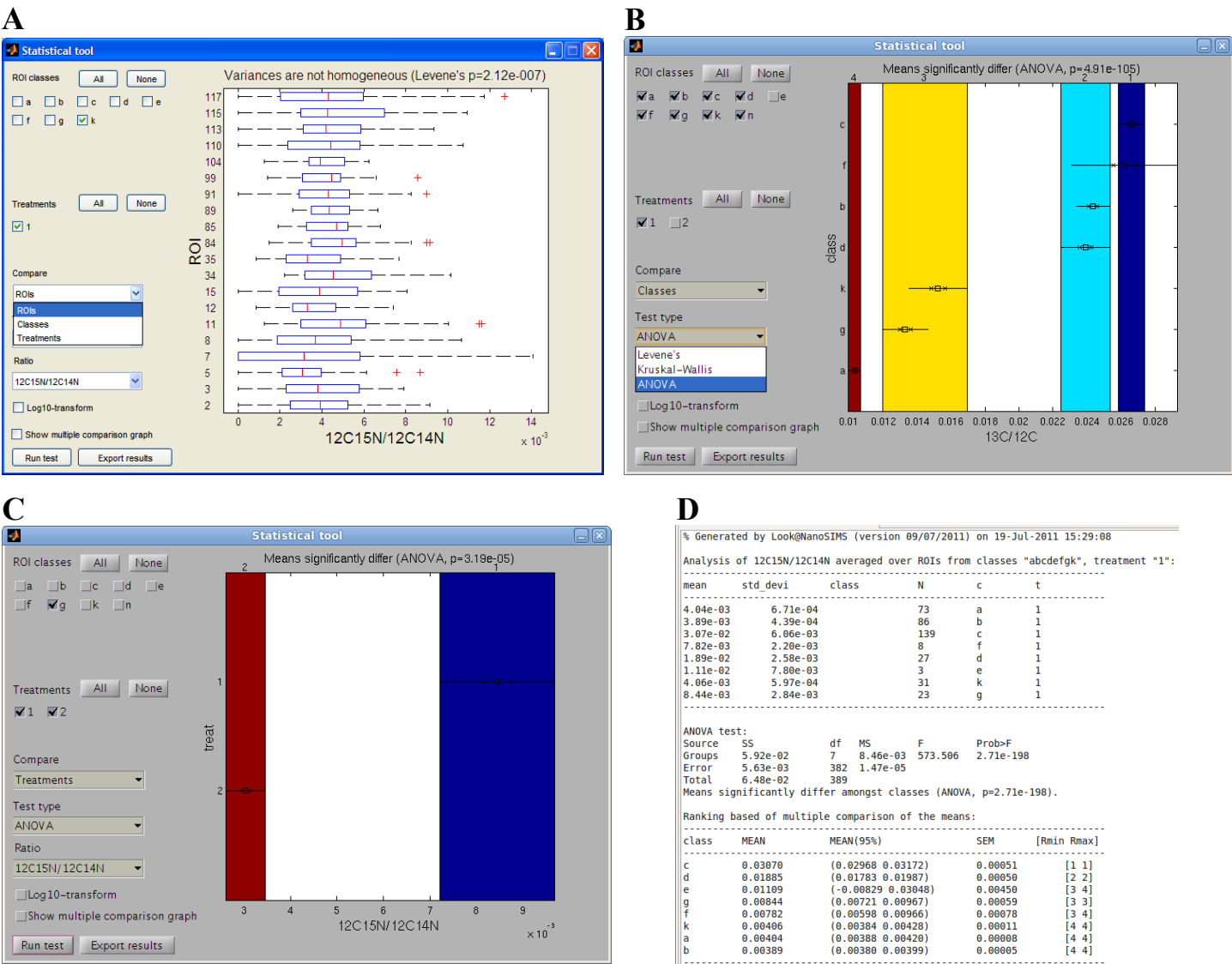
Figure S8: (**A**) Graphical user interface for interactive display of lateral profiles of masses and ratios. The profile is defined by clicking on the *Define lateral profile* button and specifying the profile's beginning and end points. The profiles are displayed separately for each selected mass or ratio (**B**) or together in one graph for all selected masses or ratios (**C**). If the thickness of the profile is set to more than 1 pixel, the value at each distance along the profile is displayed and exported as a mean +/- standard deviation of values in pixels perpendicular to the direction of the profile.

A

B

C

D

Figure S9: Graphical user interface for statistical comparison of (**A**) individual ROIs, (**B**) ROI classes and (**C**) treatments. The level of comparison is selected through the *Compare* pull-down menu, whereas the type of statistical test is selected through the *Test type* pull-down menu. When comparing individual ROIs or ROI classes, select one treatment and one or more ROI classes. In contrast, when comparing treatments, select one ROI class and multiple treatments. Data can optionally be log-transform before the comparison. Results of the statistical test are exported in a graphical (**A-C**) and text-based (**D**) format by clicking on the *Export results* button. In the graphical output, square symbols represent mean values, crosses represent boundaries of the standard error of the mean, and error bars represent 95% confidence intervals of the mean. Ranking of the mean values based on a multiple comparison procedure is graphically represented by differently coloured patches, which show boundaries of groups with significantly different means ($p < 0.05$). For example, with respect to the $^{13}C/^{12}C$ ratio (panel B), classes 'b' and 'd' do not significantly differ from each other because their symbols and error-bars lie within one patch (rank 2), but do significantly differ from classes 'k' and 'g' (rank 3) as well as classes 'c' (rank 1) and 'a' (rank 4) because their patches do not overlap. Class 'f' is significantly not different from classes 'c', 'b' and 'd' because its error-bar (95% confidence interval of the mean) overlaps with patches ranked as 1 and 2.

Figure S10: Graphical user interface for (**A**) definition of metafile instructions and (**B**) metafile processing. The type of analysis is selected through the *Tools* menu (**C**), parameters of the graphical output are specified in the *Graph / Image options* box. Metafile processing allows rapid visualization and comparison of results from multiple nanoSIMS datasets, as demonstrated in Fig. 3 and Table 2 in the main text.