BASIC WORKFLOW

- See details at: http://help.imageanalyst.net/
- Open a recording using the File/"Open Image Series/Measurement" or the toolbar button.
  - An internal loader or Bio-Formats can be used for opening files using separate menu items, or during drag-and-drop based on the preference set in the File/"Set Folder Locations and Default Open Method".
  - The internal loader is optimized for *.inf, *.nd, *.tif, *.nd2, *.lsm files.
  - Multi-dimensional files and Bio-Formats open a Multi-Dimensional Open dialog
  - Multiple files selected are merged in time, with the options for merging as channels or stage positions
  - X,Y,Z,T recordings are loaded z-projected according to the setting in the Multi-Dimensional Open Dialog Settings tab.
- Image window linkage
  - Opening multi-channel recordings result an Image Window for each channel. These windows are linked, meaning that share ROIs and are available for image processing in functions which have multiple inputs.
  - Set Image Window linkage using the toolbar button.
- View image time lapses
  - Use the toolbar button to play the time lapse. The speed of playback is set in the Edit/Preferences dialog Misc tab.
  - Use the toolbar button to synchronize the view of linked Image Windows.
  - Use right-click in the Image Window to set lookup table (LUT), scale the image or overlay Image Windows.
  - Use pipelines such as "Show three color overlay" for quick LUT adjustments and overlay.
- Process images
  - All image processing is floating point and acts on the entire time lapse.
  - Basic background subtraction, ROI and Plotting functions are available in the Tools menu, and on the toolbar.
  - All other image processing functions can be selected from the menus between "I/O" to "Special", and pipelines in the "Pipelines" menu. Functions and pipelines are controlled in the same way, by setting parameters in a list appearing below the toolbar (see next page). Use the toolbar button to process selected images with the current function or pipeline, or to open and process images from a Multi-Dimensional Open dialog.
  - A set pre-defined pipelines support many basic tasks for pre-processing fluorescence images for intensity measurements, morphological measurements and cell counting
- Save data
  - Draw ROIs by hand or by magic wand tool which works both on fluorescence and brightfield images. Automatic ROI drawing tool is available in the "Plotting" menu. An additional customizable automatic ROI drawing tool is the "Automatic ROI drawing with segmentation" pipeline in the Pipelines menu.
  - Use the toolbar button (Tools/Plot) or the advanced plotting functions in the "Plotting" menu to create plots.
  - Use image segmentation to measure morphological parameters or intensities corresponding to segments.
  - Plot data can be copied to the clipboard or saved as *.txt files
  - Plot and other measurement data also appear in the Excel Data Window when it is opened from the Tools menu.
- Export processed images
  - Processed images can be copied to the clipboard or saved as *.jpg or *.avi files.
  - The original, raw data is read only, it is never changed by the Image Analyst MKII.
- Automate the workflow using intuitive flow-chart based pipelines ( )
  - All advanced functions ( ) are available as building blocks in the pipeline window.
  - Saved pipelines will be compatible with future versions of Image Analyst MKII.
  - Pipelines can be automatically executed in all stage positions in a Multi-Dimensional data set using the button, supporting the work with microplates.

THE MAIN CONTROLS OF IMAGE ANALYST MKII

1. Start here by opening a

2. Pipelines and image processing functions: selection of these will open the parameter bar below

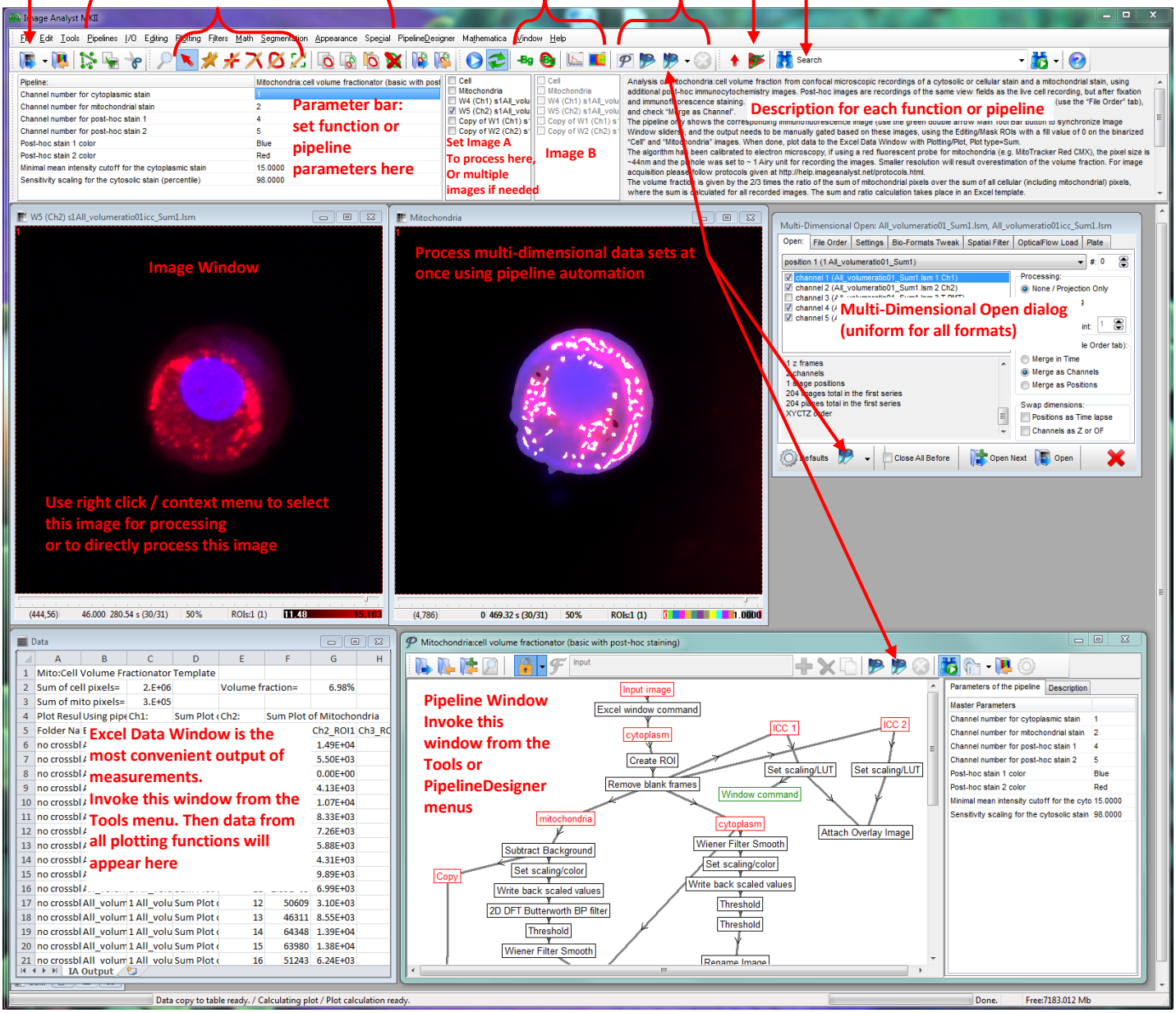
3. Process images selected as “Image A” or “Image B”

ROI functions

Basic Background Removal and plotting

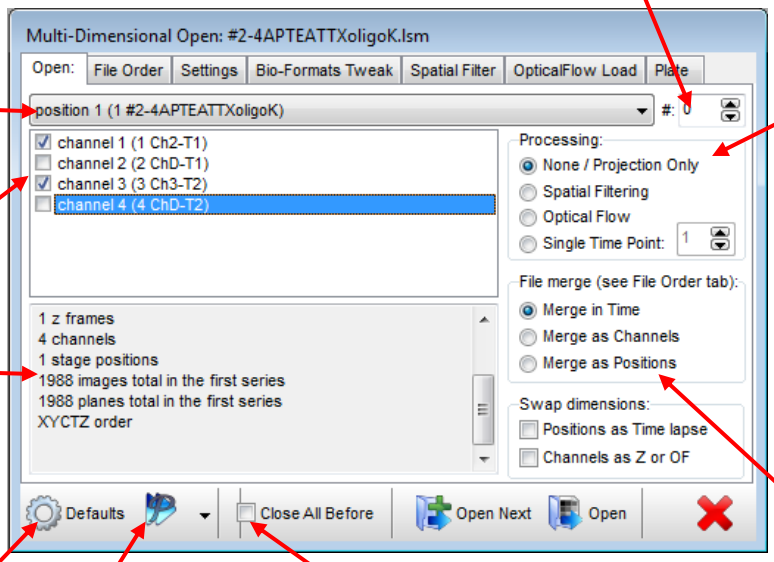
Pipeline control

Search for pipelines and functions



THE MULTI-DIMENSIONAL OPEN DIALOG

Opening Multi-dimensional files and Bio-Formats open a Multi-Dimensional Open dialog that allows selection of stage positions and channels to be loaded and also processing during or after loading.



Tag of the Multi-Dimensional Open dialog: refer to this tag from pipelines using multiple input image datasets, such as post-hoc staining recordings

Select stage position

Select channel

Information about the image dataset(s) opened.

Each loaded frame is processed according to these settings. See corresponding tabs.

The "None" setting will read raw time courses of XYT recordings and z-projection of XYZT recordings (set the type of projection or which frames to project in the "Settings" tab.

Multiple files are merged based on these settings. See also the File Order tab.

Menu to store the actual settings as default, or to set this dialog as default for pipeline processing

Pipeline automation button:

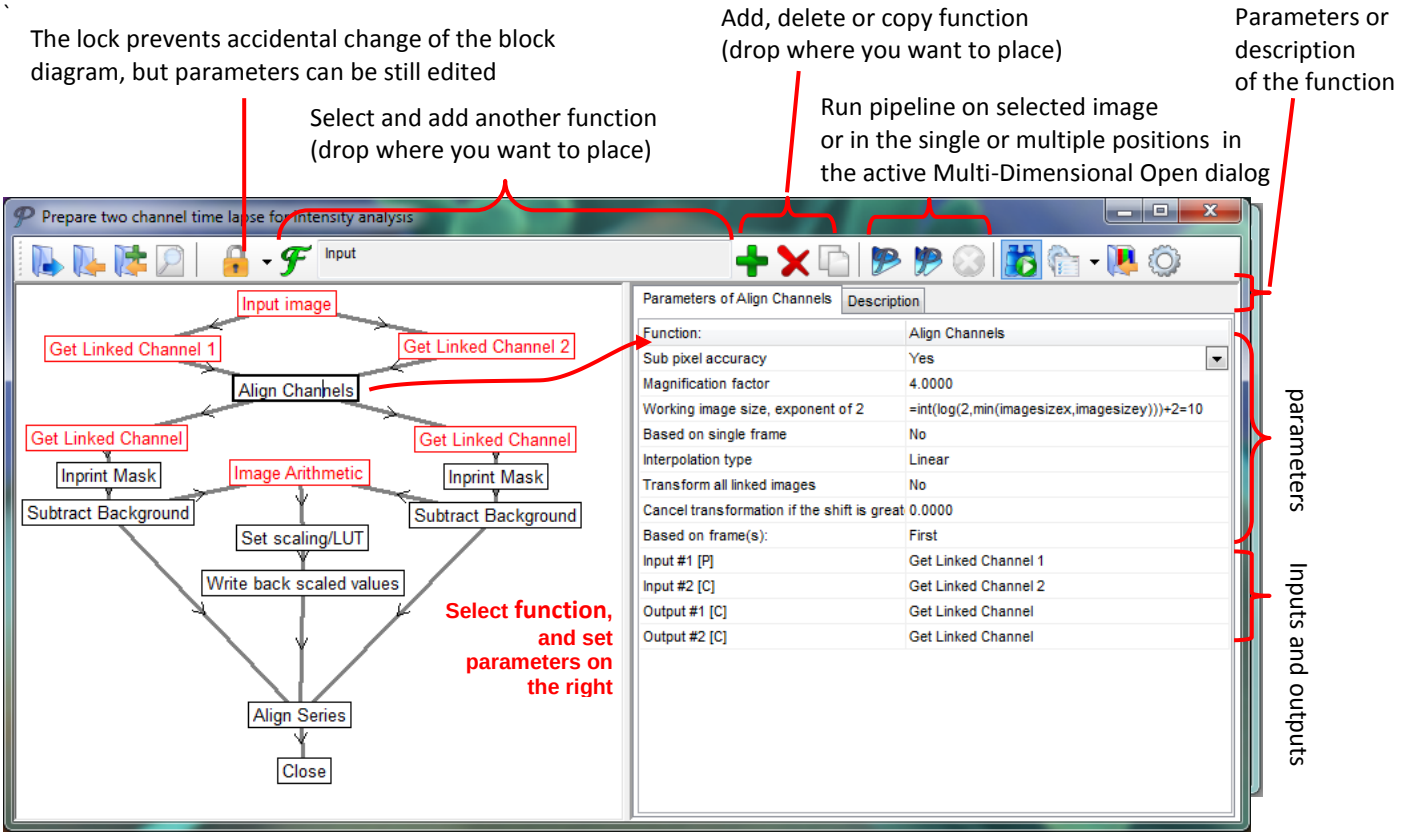
Load and process single or multiple positions using the pipeline selected from the main menu

Setting this option will close all Image and Plot windows when the *Open* or *Open Next* buttons are pressed.

- Use the "File Order" tab to add, remove or reorder multiple files.
- Use the "Settings" tab to open specific ranges of frames in t or z dimensions and to select how to z-project XYZT recordings.
- Use the "Plate" tab to reassign multiwall plate well names to each position.
- The "Spatial Filter" and "Optical Flow" tabs offer specific during-loading image processing.

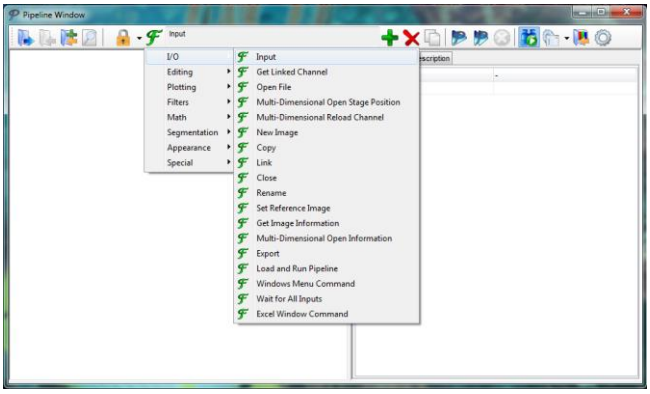
THE PIPELINE WINDOW

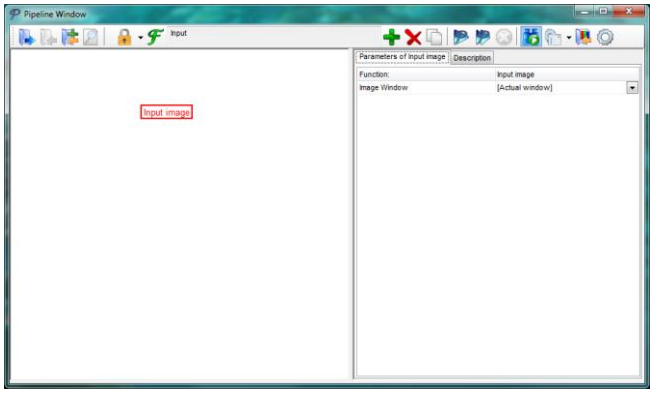
- Select a pipeline in the Pipeline menu. The selected pipeline can be used in the main parameter bar and executed by the , or buttons. In order to edit the pipeline, the pipeline window can be opened using the button. All built-in pipelines can be modified.
- An Empty Pipeline window can be opened with the PipelineDesigner/New Pipeline Window menu point.
- To load an arbitrary pipeline, use drag-and-drop, or open an empty Pipeline Window and press the open button .



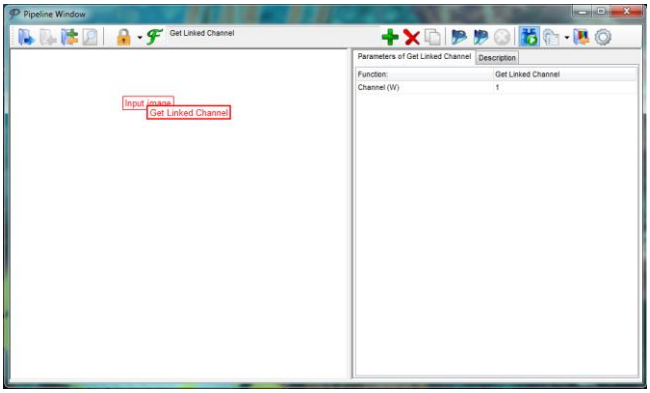
RULES OF PIPELINE EDITING:

- Always start with an input image.
- Select functions to add by pressing the $\mathcal{F}$ button.

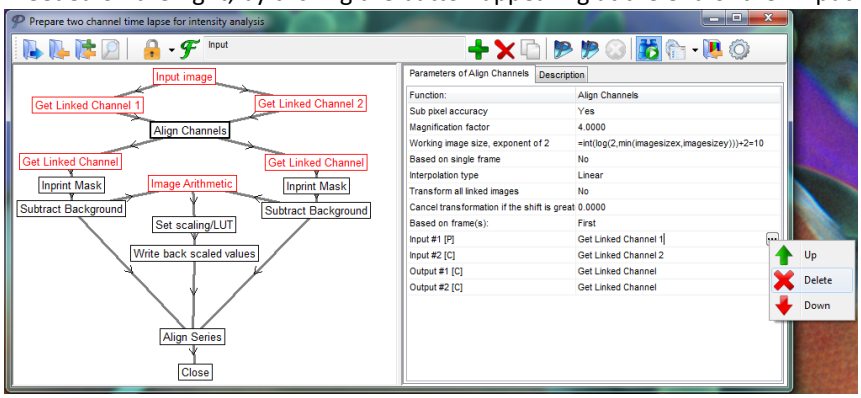




- Place new functions in an empty area then drag-and-drop onto their inputs.



- Red functions are inputs, seek for certain channel or create a new image.
- Black functions are in place operations; Only one black function can be immediately downstream of any function.
- Green and blue functions do not produce output images, so these are dead ends in the flow chart.
- Placed functions can be freely renamed for more intuitive diagrams.
- Reorder the inputs if needed on the right, by clicking the button appearing at the end of the “Input #” entry.

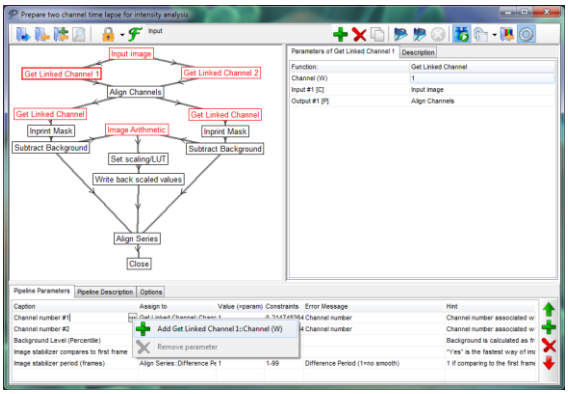


- Pipelines are parallel processed, and the pipeline design has to avoid conflicts resulting from simultaneous manipulations of Image Windows. Furthermore the timing of parallel branches is not predictable, therefore certain designs may result in variable order of data output. Pipeline processing starts simultaneously in all Inputs.

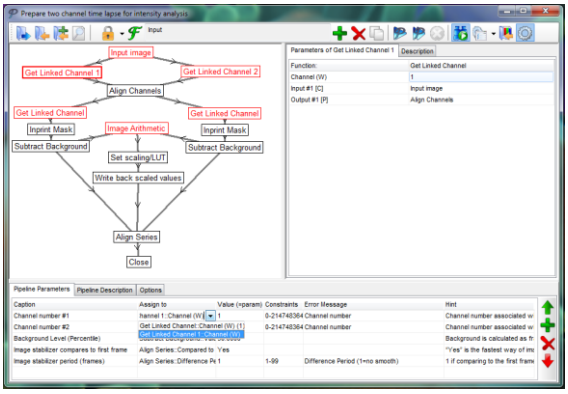
ADDING MASTER PARAMETERS TO PIPELINES

Pipelines can have master parameters (these are the parameters shown in the main parameter bar of Image Analyst MKII) that relate to one or more function parameters within the pipeline.

- Press the button in the toolbar of the (unlocked) Pipeline Window.
- Select a function parameter that you want to use as a pipeline parameter.
- Click the first empty cell in the Caption column in the bottom.
- Click the button at the end of the Caption edit and Add the parameter.



- Multiple parameters of the same type (integer, real, Boolean or text) can be added to each pipeline parameter.
- To switch between linked parameters, use the picklist in the Assign To column.



- The numerical value of a pipeline parameter can be assigned to a function parameter by expressing it using a mathematical formula started with an equation mark typed in the parameter of the function, and referring to the associated pipeline parameter as “param”. Thus =2*param will pass the double of the value of the pipeline parameter to the given function. See details on the math parser (evaluator) in the Help menu or (see http://help.imageanalyst.net/workflow_parser.html).