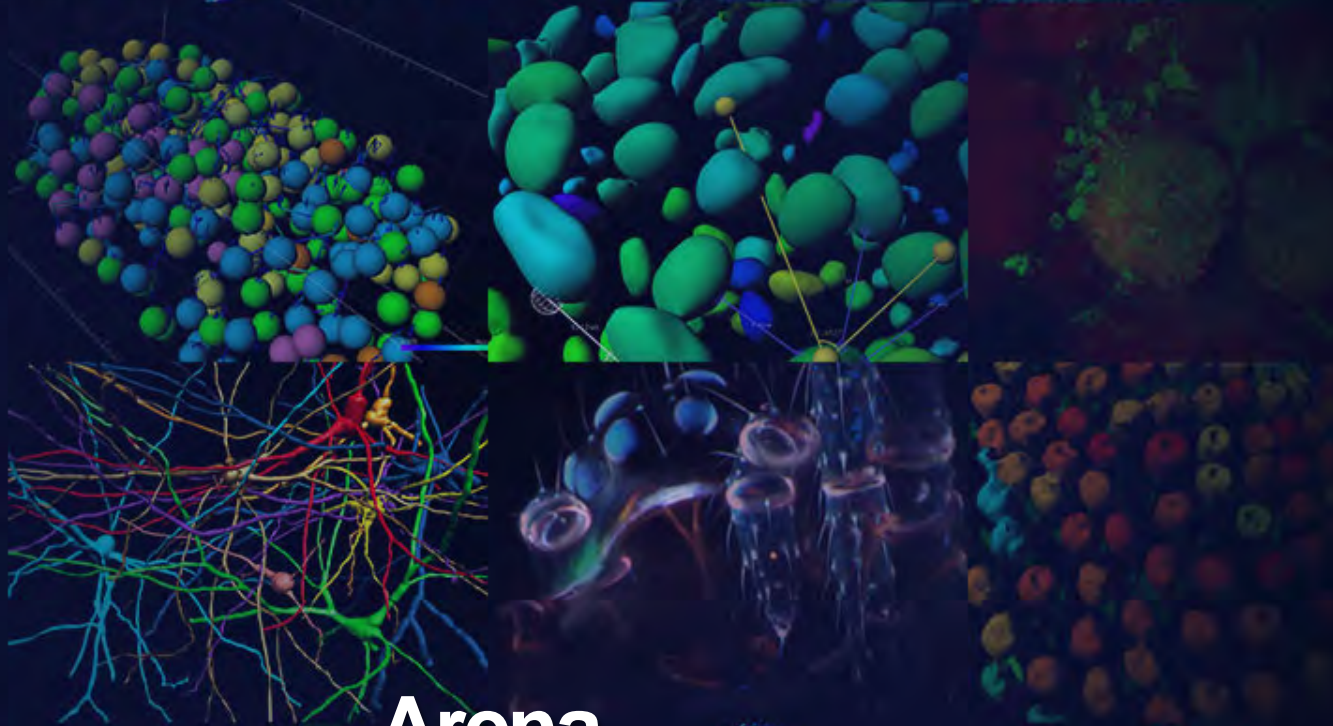# BITPLANE

an **Oxford Instruments** company

Arena

Managing, grouping and searching images, controlling Batch runs, compiling results for Vantage

BITPLANE
an **Oxford Instruments** company

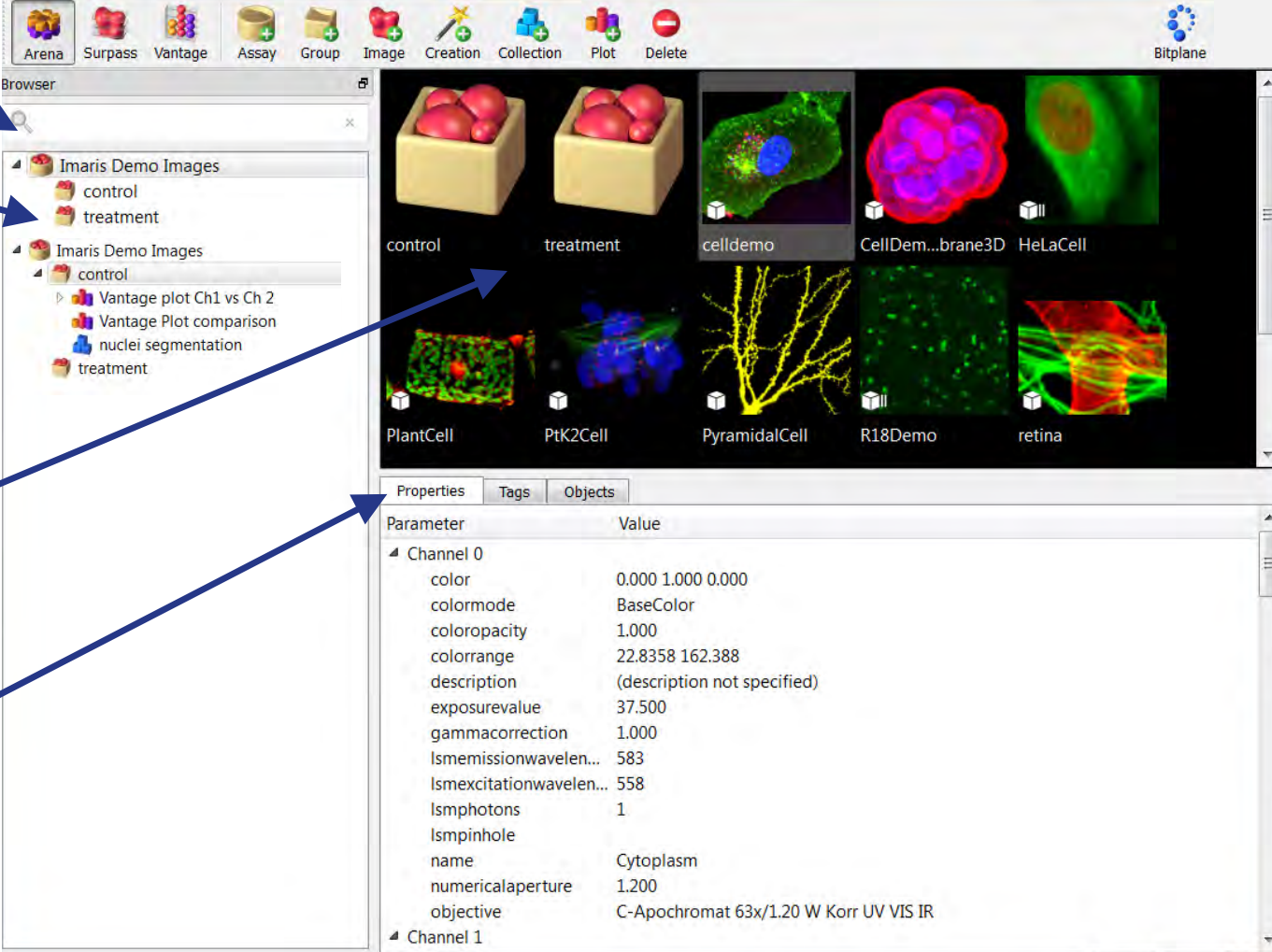**Search field** (files, tags…)

Arena tree

provides direct access to all the components of your experimental data including creation parameters, Vantage plots and (batch) results

Arena View

displays contents of the current tree location or any search results

Image details

displays object's properties, tags and surpass objects

Arena item	Icons	
Assay	Empty	Filled
Group	Empty	Filled
Image		
Creation parameters		
Collection	Batch Collection	Manually created Collection
Vantage Plot		

Arena (typical) workflow

1. Create an Assay

2. Create groups

3. Add images to groups

4. Analyze/segment your image(s)

5. Use creation parameters to create a Batch run

6. Collections (automatically or manually created) contain analysis results

7. Create a Vantage plot for comparing results and do hypothesis testing
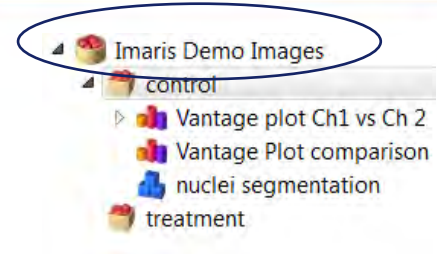
Arena items: Assay

❑ Assay is the top level Arena item.

❑ It should be used to handle a large series of similar data. It may contain a large amount of experimental image data and any associated information.

❑ To start working with Imaris, you must first create an Assay.

❑ The Assay can hold all Arena items: Groups, Images, Creation Parameter, Collections, Vantage plots.
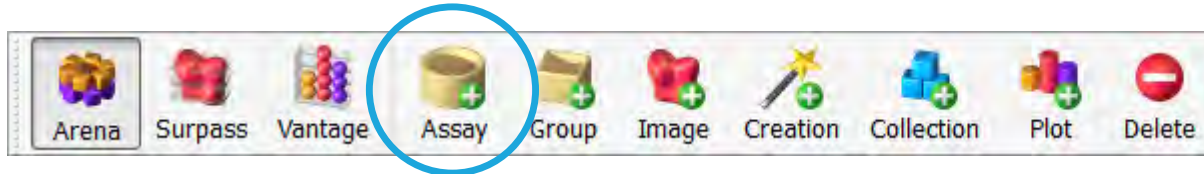
1. To create an Assay, select the **Add Assay** icon.
2. Enter a descriptive name and a new Assay is created in the Arena tree.
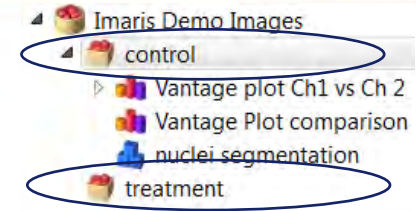
□ The *Group* item is a child of Assay.

□ A Group item helps you keep everything organized by allowing you to gather corresponding items together.

□ A Group can contain 2D, 3D and 4D images or any other Arena item.

□ A typical Group consists of many images, creation parameters, Batch results, and/or Vantages plots items. Within the Group, each Arena item is labeled with a special icon. The icon graphically indicates the type of item.
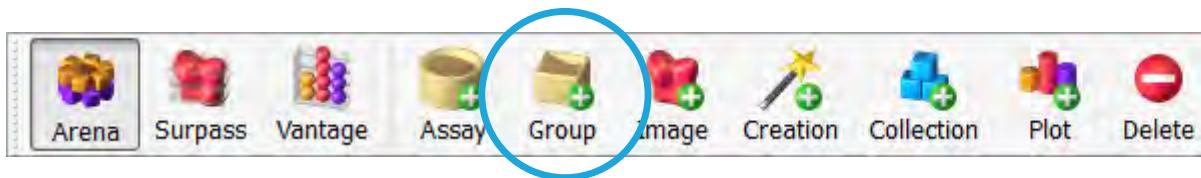
1. To create a Group, select the **add Group** icon.
2. Enter a descriptive name and a new Group is created both within the Arena tree and the Arena view.

Arena items: Image

Images can be added to the Assay and/or to Group(s)
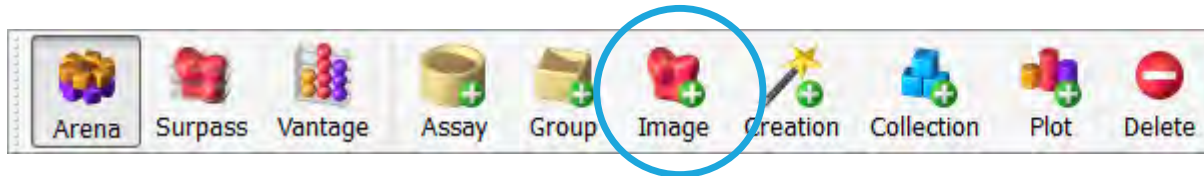
1. Select the **add Image** icon.

2. Browse your folder(s) containing images.

3. Select one or multiple images using standard multiple file selection commands.

Directly Read More than 40 Microscopy File Types

- Andor: Multi-Tiff (Series) (*.tiff, *.tif)
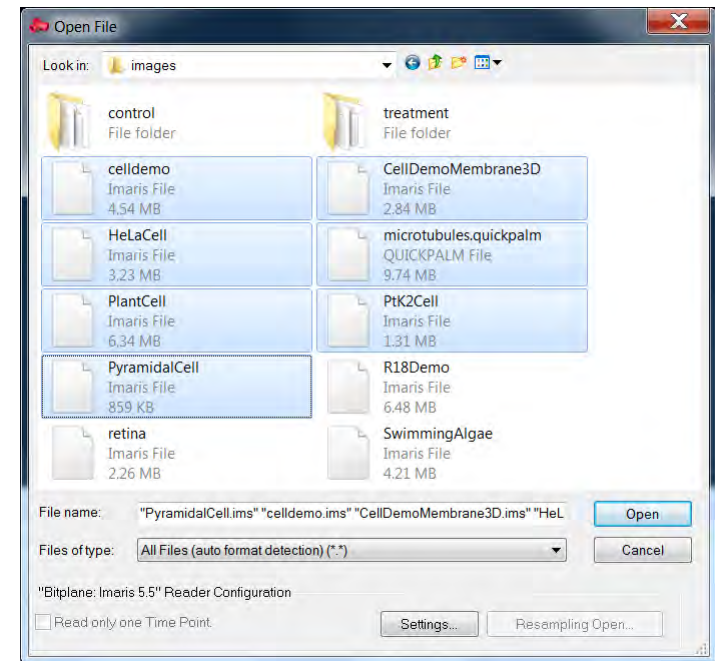- Andor: iQ ImageDisk (*.kinetic)
- Applied Precision DeltaVision (*.r3d, *.d3d, *.dv)
- Big Data Viewer (*.xml, *.h5)
- Biorad MRC 1024, 600 Series (*.pic)
- Biovision: Ivision (*.ipm)
- Biovision / Scanalytics: IP Lab (series) (*.ipl)
- Bitplane: Imaris 2.7, Imaris 3, and Imaris 5.5 (*.ims)
- Bitplane: Imaris Scene File (*.imx)
- BMP (adjustable file series) (*.bmp)
- Gatan Digital Micrograph (*.dm3)
- Hamamatsu/Compix SimplePCI (*.cxd)
- IMOD binary file (*.imod, *.mod), object scene file
- IMOD MRC file (*.mrc, *.st, *.rec)
- 3i Slidebook (*.sld)
- Leica Image Format LIF (*.lif)
- Leica Vista LCS (*.tif, *.tiff, *.lei, *.raw)
- Leica Series (*.tif, *.tiff, *.inf, *info)
- Leica TCS-NT (*.tif, *.tiff)
- Molecular Devices: Metamorph Stack (*.stk)
- Molecular Devices: Metamorph ND (*.tif, *.nd)
- Micro-Manager Image 5D (*.tif *.tiff *.txt)
- Nikon ICS – "Huygens compatible" (*.ics, *.ids)
- Nikon Elements ND2 (*.ND2)

- Olympus Cell^R (*.tif, *.tiff)
- Olympus FluoView (*.tif, *.tiff)TIFF
- Olympus OIB (*.oib)
- Olympus OIF (*.oif)
- Olympus OIR (*.oir)
- Olympus VSI (*.vsi, *.tif)
- Open Microscopy Environment Tiff (*.tiff, *.tif)
- Open Microscopy Environment XML (*.ome)
- Perkin Elmer: Ultraview (*.tim, *.zpo)
- Perkin Elmer: Volocity / OpenLab LIFF (*.liff)
- Perkin Elmer: Volocity / OpenLab RAW (*.raw)
- Prairie Technologies (*.xml, *.cfg, *.tif, *.tiff)
- Quick PALM (.quickpalm, .tif)
- TIFF (adjustable file series) (*.tiff)
- TILL Photonics: TILLvisION (*.rbinf)
- Zeiss Axiovision (*.zvi)
- Zeiss LSM410, LSM310 (*.tif, *.tiff)
- Zeiss LSM510, LSM 710 (*.lsm)
- Zeiss CZI (Series) (*.czi)
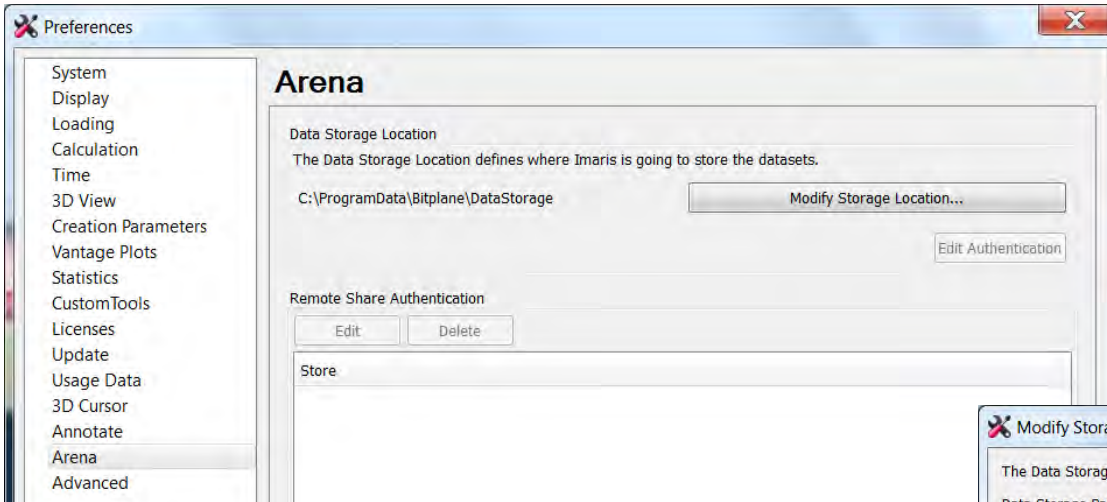
Arena: Data Storage

Default Data Storage location:

PC: C:\ProgramData\Bitplane\DataStorage
Mac: \Library\Bitplane\DataStorage
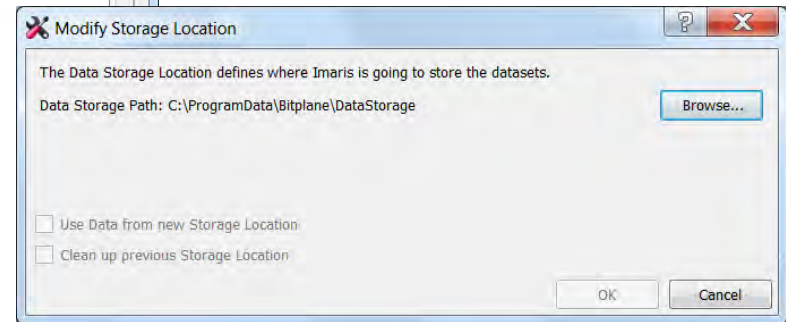
The Store/Restore Arena options are only available if the Data Storage location differs from the default one.
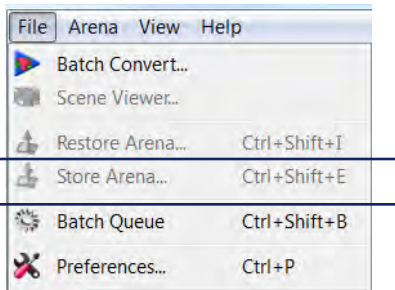
Using **Store Arena** you can copy image files to the data storage location that has been set by the user under Arena Preferences. This enables your Arena data files to be stored on a range of local and remote locations. This includes HDD or SSD drives, and removable data storage devices such as USB memory sticks. When using network server locations you will require authentication to access the network server.

Arena: Data Storage

Note that Store and Restore functions are not available if they have been disabled under Imaris Administrator. This is to prevent overwriting files that may be used by more than one user, for example, image files saved on a remote, network server location that may be accessed and modified independently by different users.

File	Arena	View	Help	
▶	Batch Convert...			
	Scene Viewer...			
⚒	Restore Arena...		Ctrl+Shift+I	
⚒	Store Arena...		Ctrl+Shift+E	
⚙	Batch Queue		Ctrl+Shift+B	
✖	Preferences...		Ctrl+P	
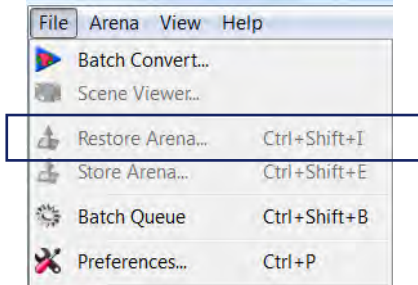
With **Restore Arena** you can restore image files in Arena View to the time of the last save. For example, if you have deleted, or modified a file by accident.

Note that when the Restore Arena function is used, all image data in Arena will be overwritten as the image data will be returned to that of the previous save.
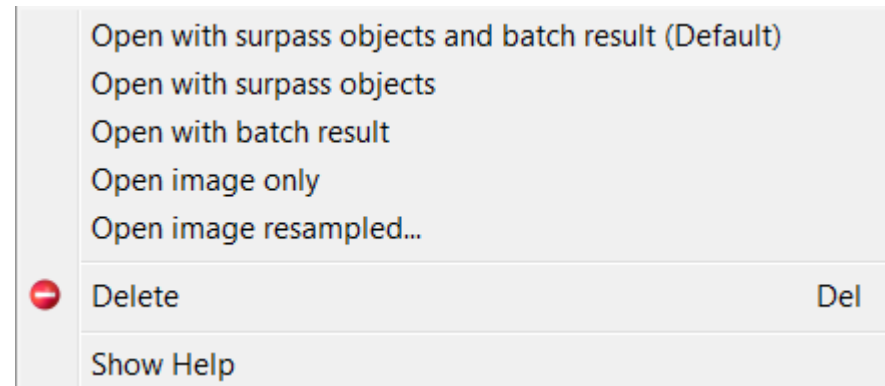
❑ To **copy** an item to a new location, select the item within the Arena view and then simply **drag and drop** the item into another tree location. The item's icon changes to show a plus (+) sign indicating that you are in drag mode; a minus (-) sign will appear when a drop is not allowed.

☞ *Please note that when you drag and drop an item to a new tree location within the Arena, that item will be copied, not moved.*

❑ If the item dragged is a whole Group, Imaris will automatically recognize its contents and add all its files to the new chosen location. Any hierarchy within the items that you drag and drop will be retained.

❑ You can drag as many Groups or items as required, and you can add them to any level of the Tree structure. The insertion is indicated and highlighted within the tree. The amount of indentation indicates the level at which an item is located within the tree hierarchy.

❑ To **move items** you can use **drag and drop** in combination with the **Control key (+CTRL)**.

Opening Data Sets

Arena

- Double click for direct opening within Surpass, or right+click for a contextual menu:

Open with surpass objects and batch result (Default)
Open with surpass objects
Open with batch result
Open image only
Open image resampled...
⊖ Delete   Del
Show Help

Surpass

- Click on the File menu…
    Open

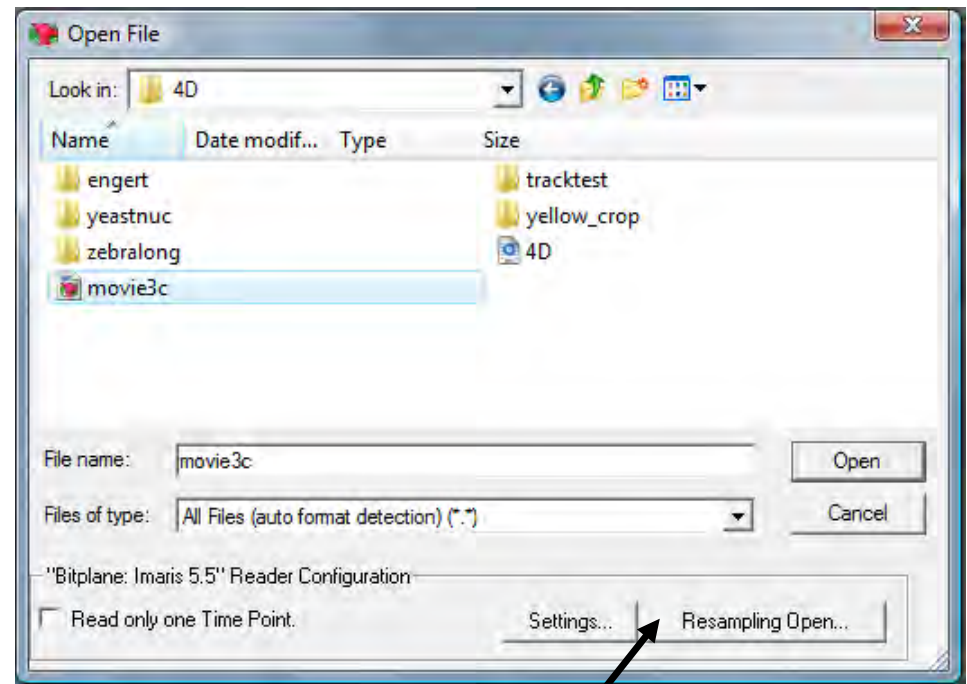# Resampling Open

Under the <u>File</u> menu, choose <u>Open</u>, click <u>Resampling Open</u> Button

- Why – To reduce the image file size before reading the file into memory

- Smaller Files = Better Performance

- Eliminate regions with no data

- Resample the data if oversampled

- Eliminate unneeded channels

Under the <u>File</u> menu, choose <u>Open</u>, click <u>Resampling Open</u> Button

- Resample in X, Y, Z, T or Channel

- Crop in X, Y, Z, T or Channel
  - Can use mouse to resize XY limits

- Change contrast: drag right mouse button in image preview

- Preview image with Z and T sliders

  Original data size shown on top
  New data size shown on bottom
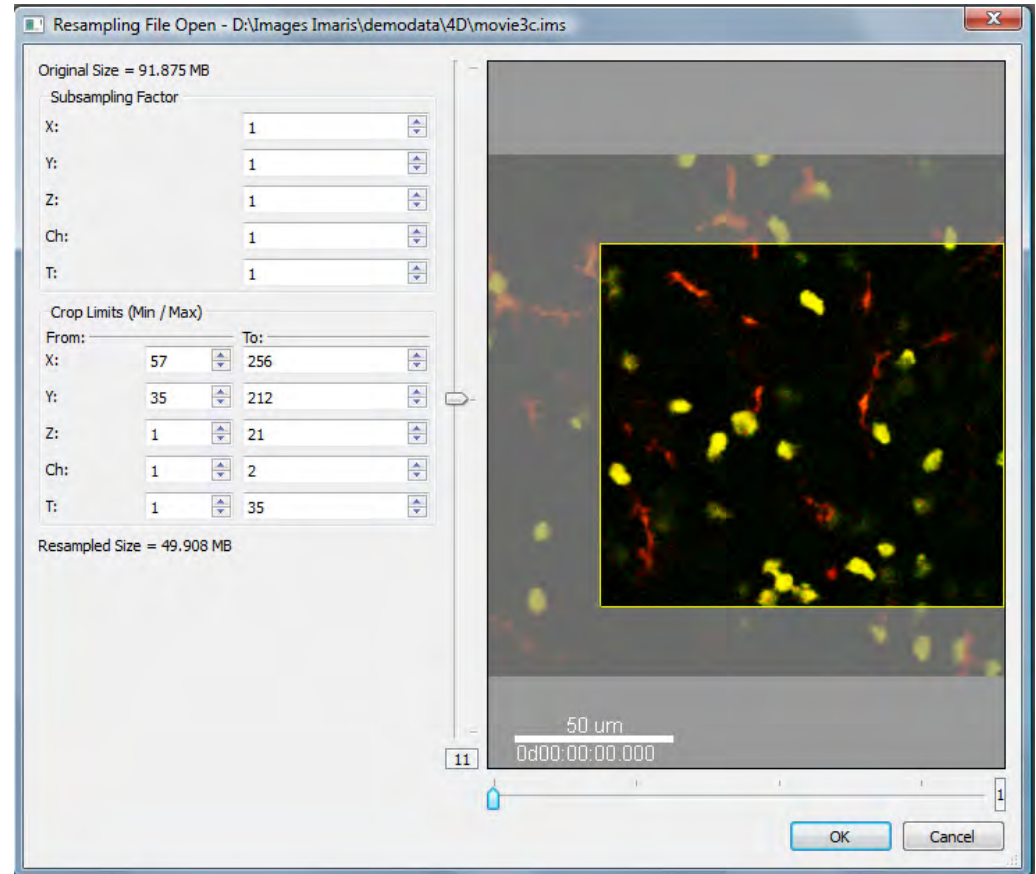
Under the <u>File</u> menu, choose <u>Open</u>, select a file, click <u>Settings</u> Button

- Access to special settings for selected file types

- Settings button will only work when user single-clicks on applicable file types

- Applies to Leica *.lei, Leica *.lif, Zeiss *.lsm and Andor IQ Disk *.kinetic as multi-image format, Imaris *.ims, *.TIF file series, and *.BMP file series
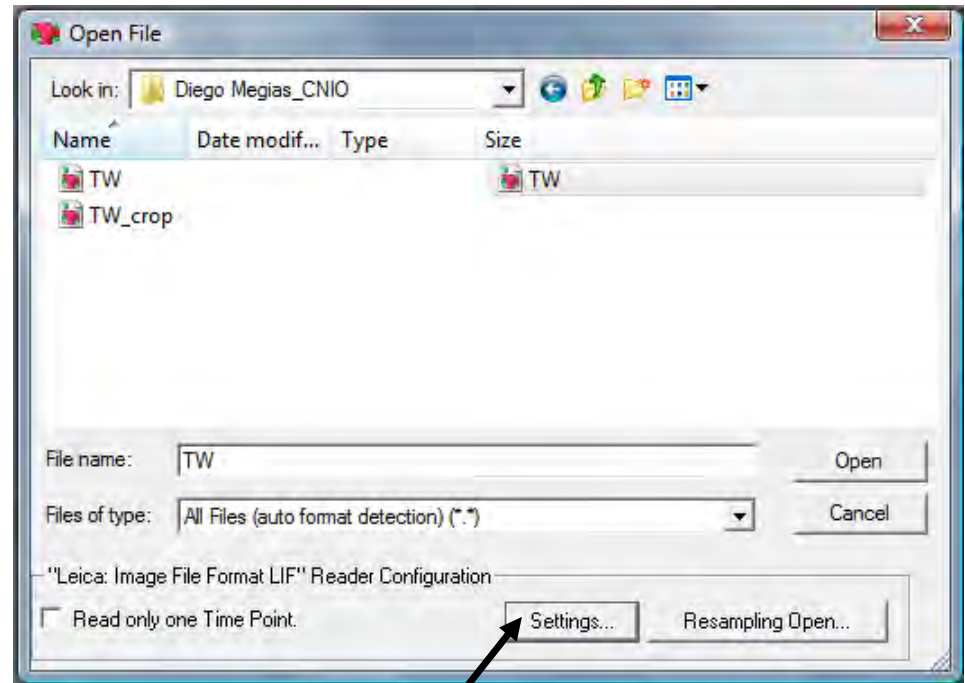
File Open Settings: Leica *.lif

- The *.lif file can contain any number of 2D images, 3D images, 4D images, projections, or movies.

- Check Thumbnail Preview to show a thumbnail for each image.

- Click on each image in the list and the properties area below will tell you details about the particular image so you can choose the right one.

Imaris will always read the first image, if the user does not choose another one via "Settings".

File Open Settings: *.tiff / *.bmp
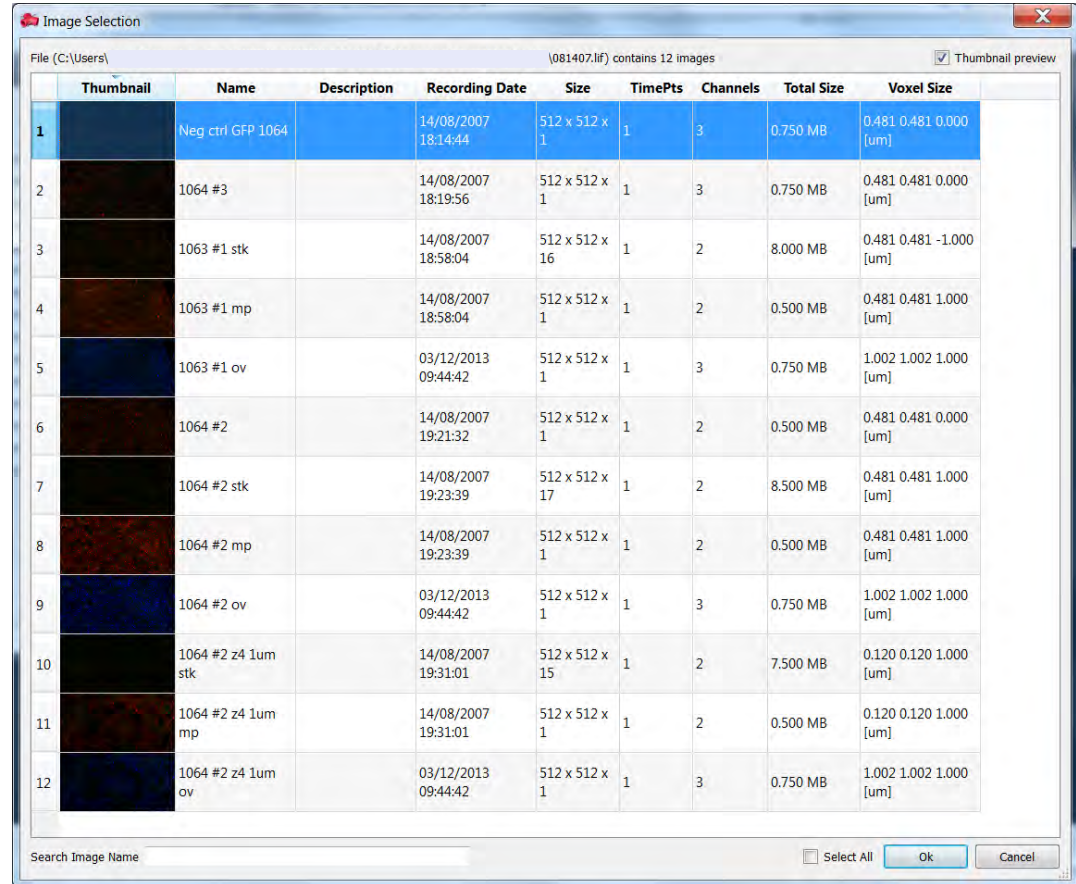
File Names with Delimiter

- Used if multiple numeric sequences are annotated in the file names
- Non-numeric "delimiters" in file names assign each sequence to a dimension
- Preview arrangement of how the files will be read into Imaris

File Names without Delimiter

- Used if file names don't give a hint about number and sequence of files per dimension
- User sets the number of files per dimension, and the order of dimensions in the series
- Preview arrangement of how the files will be read into Imaris
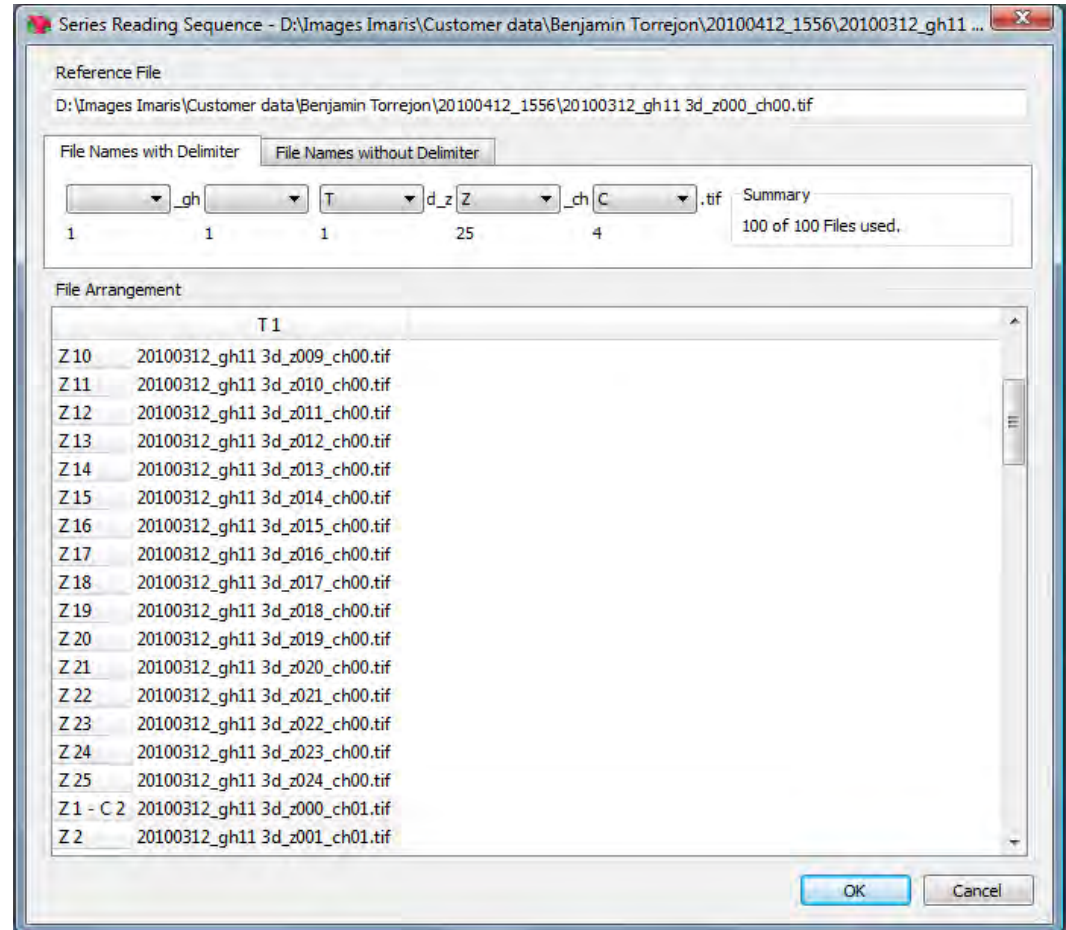
Creation Parameters contain the batch protocol for calculating Spots, Surfaces, Cells, and Filaments. Can be executed on a group of images.

❏ Possibility to Copy the creation parameters between Groups to apply the same creation parameters in multiple Groups

❏ To **copy** the creation parameters from one Group to a new one, select the creation parameters item that you want to copy and then **drag and drop** it into the newly chosen Group.

❏ Right-click options

|	
⚙	Run Batch
⚙	Run Batch including Sub-Groups
⬇	Export...
	Rename F2
⛔	Delete Del
	Show Help
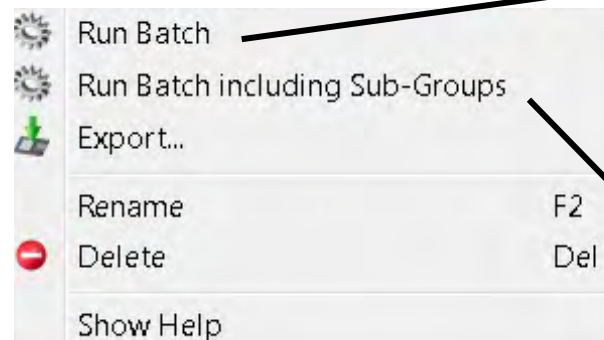
Imaris applies the selected creation parameters set (that you created for the chosen image) to all images within the Group.

This option recursively applies the creation permeates to all the images of the chosen Assay/Group and all its subgroups.

Arena items: Creation Parameters

To be able to apply the creation parameters to all images within the Group you
need to set them up.

- ❑ Identify the most-representative image of the whole group.

- ❑ Double click on the image thumbnail to open it in the Surpass view.

- ❑ Complete an appropriate Creation Wizard (Cells, Filament, Spots
  and Surfaces) and create the required objects.

- ❑ Store the Creation parameters in Arena and/or to Favorites in the
 Creation (Rebuild) tab
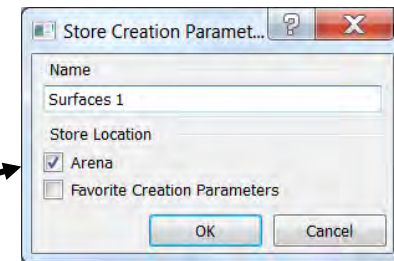
To import creation parameters files (*icpx) generated by the Export command, use the
Creation icon.

☞ Under the File/Preference/Creation Parameters the Export and Import buttons provide
an easy way to exchange creation parameters between different users or Arena groups.

Arena items: Collections

Object Collection contains calculated objects (e.g. Spots) and statistics from the images in the group.
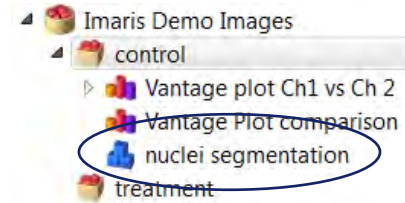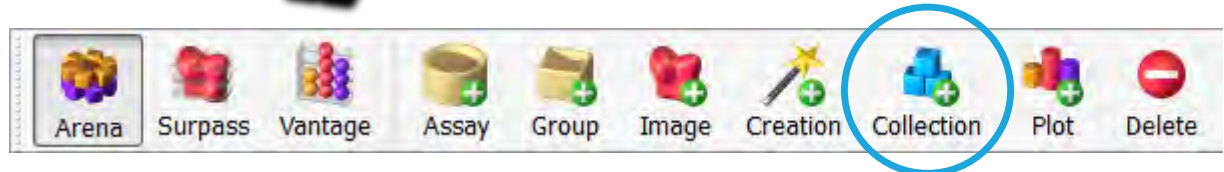
- o **Batch Collection**
  - As soon as you press the *Run Batch Job* option Imaris generates a new Batch Collection item
- o **Manually created Collection**

Should be used:

- when automatic image segmentation cannot be applied. The collection contains manually created objects as an alternative input to the generate Vantage Plot comparison items.

> ☞ To **integrate** surpass objects within the Manual Collection item from a "Store" or "Store As" image, **select the object icon from the Image Objects Tab** and then **drag and drop** it onto the chosen Manual Collection item.
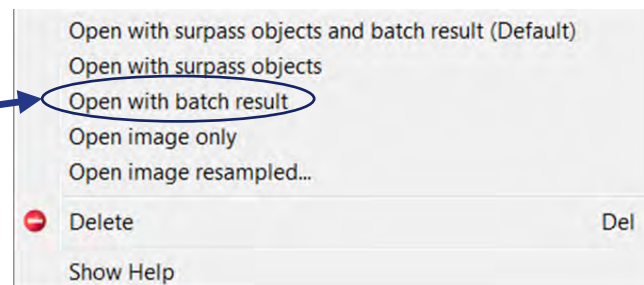
- for combining manually created objects with the automatic Batch Collections within one Vantage Plot item

The following status icons are used during the Batch Job execution process :

1.   *Completed* - the image is segmented and analyzed

2.   *Processing.* The proportion of the complied Bath Job is indicated by an icon displaying corresponding circle segments

3.   *Failed* - the image(s) have not been segmented and analyzed

After the creation parameters have been successfully applied to an image, the icon in the bottom corner of the image thumbnail shows that the batch job is completed

To validate the Batch results, double-click on the image or use Right+Click

Open with surpass objects and batch result (Default)
Open with surpass objects
Open with batch result
Open image only
Open image resampled...
Delete   Del
Show Help

Within the Surpass tree the regular object icon has been changed. A new icon **Objects Locked**, 🚫 superimposed on the object type icon, indicates that the required objects were batch created and therefore **you will not be able to manually modify them.**

☞ *These objects do not have an Edit tab. Under the Creation tab, the Duplicate and Rebuild button provides two functionality:*

1. *To copy the Batch created objects to a new Surpass Tree object*
2. *To re-enter the Creation Wizard and use previous parameter settings and processing instructions as initial values.*

❑ If you are not pleased with the Batch segmentation results and you find it necessary to manually modify them, **all the changes that you make will not be reflected in the Batch Collection item**.

❑ To **modify the batched processed image** you can open it and start a <u>new creation wizard</u>. As you proceed with the new creation wizard, in each step all creation parameters are initially set to match the batch creation parameters' values. Adjust them and modify all parameters as necessary to obtain new segmentation results.

❑ These newly segmented objects **must be saved using either the Store or Store as** option. As Imaris does not allow the modification of batch created objects, these newly segmented objects have the **same characteristics as Manually created** ones.

❑ Once the manually created changes have been saved, within the Arena, the image thumbnail is updated. In its corner, a small overlaying object icon appears and indicates that the created objects has been assigned to an image.

❑ To **combine and compare** these modified objects with the automatic Batch Results Collections, follow the procedure of the **Manual Collection.**
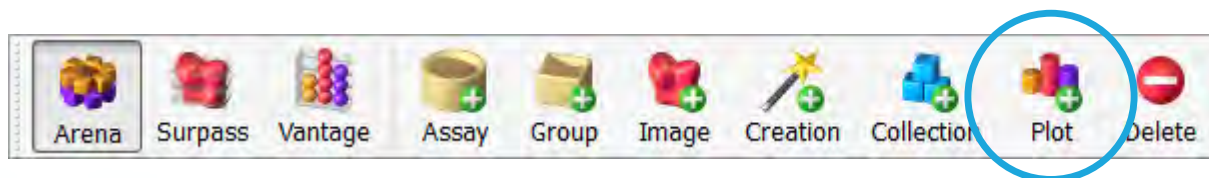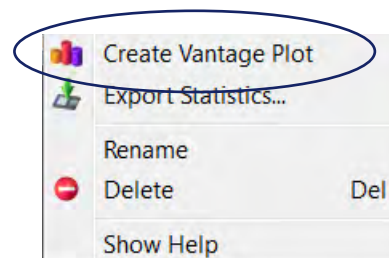
Arena items: Vantage Plot
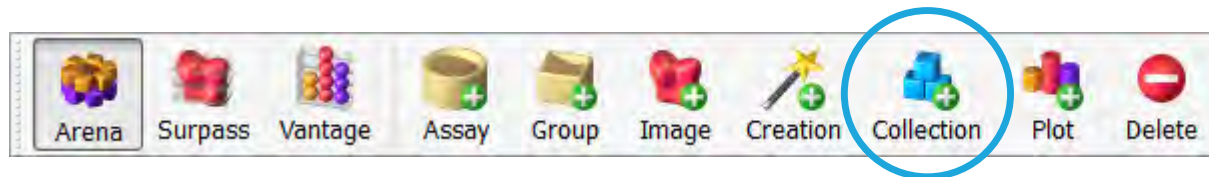
*The Vantage Plot contains the settings to display one or more collections of objects as plot in Vantage.*
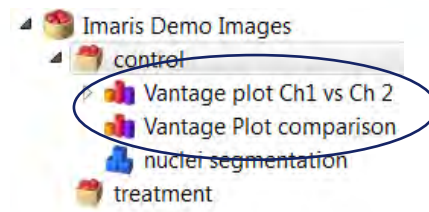
2 ways to create a Vantage plot:

1. To visualize the **Batch created objects**, right+click on a Collection

2. To create a Vantage Plot item, add *Plot* from the menu bar – useful **to compare Batch Collection results**

- To compare collection results, drag and drop the collections into a Plot item

- Right+Click on the Plot item to open the result in Vantage
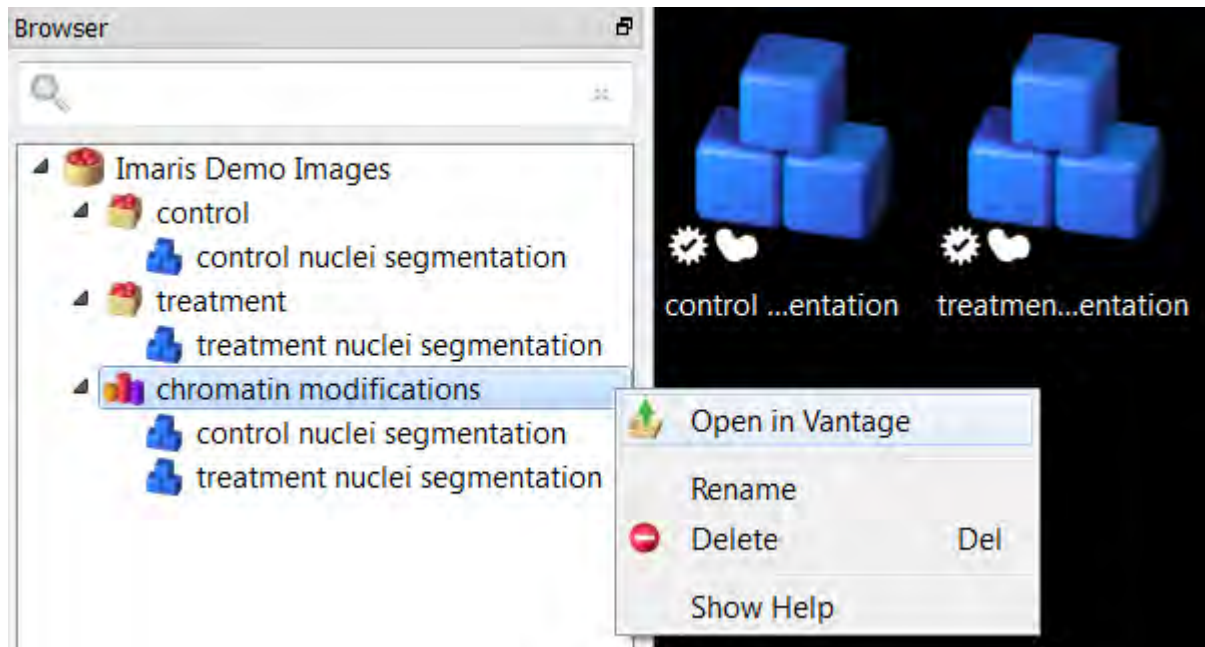
☞ *Within one Vantage Plot item you can drag and drop as many Batch Collection items as you wish to compare.*

1. Create an Assay, and 2 groups within the Assay called "Control" and "Treatment".

2. Add images in the respective folders (found in the Bitplane image folder).

3. Add manually a Creation Item (icpx file found in "Control" Bitplane image folder).

4. Apply a Batch run on the Control images.

5. Do the same as above on the Treatment images.

6. Create a comparative Vantage plot of the 2 collections based on intensity Sum of Channel 1.