The first step in any analysis is to set up a database for the movie. This database contains links to the raw image, links to all the processed and stored result files, and the parameters used for image processing.

## **Bio-Formats**

Image files can be directly imported into movie databases for analysis. This functionality **requires** the Bio-Formats loci\_tools.jar library to be installed. Details and download URLS can be found at <a href="http://www.openmicroscopy.org/site/products/bio-formats">http://www.openmicroscopy.org/site/products/bio-formats</a>.

## **Import movie using Bio-Formats:**

After clicking on this button you will be asked to select a file containing the movie. If **Uncompress channels as series of TIFF files** is checked, you will be asked to specify a folder where to uncompress the raw data. You will be asked to specify a path where to save the movie database MAT file as well as the movie analysis.

Once the movie is imported into Bio-Formats, the movie interface will be reloaded in preview mode.

## Channels

This allows you to setup a movie database by selecting folders containing the images for each channel of the movie. Each channel (wavelength) must be in a separate folder, with one file per frame (time point). Note that all channels should have the same number of images, and their images should all be the same size.

#### Add channel:

This allows you to select a directory containing images from a channel of the movie.

#### Delete

This allows you to delete the selected channel from the channels list.

### Advanced channel settings (optional):

This allows you to enter additional channel-specific information like the excitation and emission wavelengths and the exposure time.

# **Output Path:**

This allows you to specify the folder where the results of the processing will be stored. Note that if multiple movies are being processed, each movie should have its own output folder or the results will be overwritten.

# **Movie Information (optional):**

This allows you to enter metadata specific to the movie (see Advanced Channel Settings for channel-specific metadata). Metadata is usually optional but **can be required by some analysis packages**. For example, creating scale bars or time stamps in subsequent steps of the Tracking Package will require pixel size or time interval data respectively.

## Pixel size:

The pixel size of the camera in the object domain, i.e. the physical pixel size divided by the magnification (in nm).

## Time interval:

The frame rate of the movie (in s).

# **Numerical aperture:**

The numerical aperture of the microscope lens.

## Camera bit depth:

The bit depth of the camera (in bits).

# **Notes (optional):**

This allows you to enter notes describing the condition, experiment number, the date, etc. - anything you want!