KiT2 Manual

Written by Christopher Smith (chrissmith.software@gmail.com)
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Contents

Technical requirements

Compatible MATLAB versions: R2015b onwards.
We recommend that you download the latest version of MATLAB, however any version later than and including MATLAB R2015b will successfully run KiT2.
KiT2 has been tested in the following versions of MATLAB (as on 04/03/2018):

R2017b, R2017a, R2016a, R2015b

Getting started

Add KiT_2.x.x to MATLAB folder, and add to path

1. If you haven't already, move the KiT2 software to your MATLAB home folder.
2. Next, ensure that MATLAB knows that the KiT2 software exists. To do this, open MATLAB, and within the *Current Folder* panel, locate the KiT2 folder, right-click, and select *Add to Path -> Selected Folders and Subfolders*.
3. To save KiT2 to your MATLAB path permanently, type `savepath` into the Command Window panel of MATLAB and press enter.

If you're not familiar with MATLAB's coding language, become familiar!
YouTube contains numerous videos demonstrating how to use MATLAB, plus check out MathWorks for tutorials.

Quick start guide

To make a new jobset file:

```
jobset = kitGUI
```

This generates a GUI within which channel information, spot detection, tracking, chromatic shift correction, and intensity measurement options can be provided.
To make basic edits to an already generated jobset:

```
jobset = kitGUI(jobset)
```

To run a jobset:

```
kitRunJobs(jobset)
```

Alternatively, to run a subset of movies, say movies 2, 4 and 5, within a jobset:

```
kitRunJobs(jobset,'subset',[2 4 5])
```

Once the tracking is complete, the resulting job can be loaded:

```
jobs = kitLoadAllJobs(jobset)
```

Individual jobs, say for movie 3, can then be accessed by typing `jobs{3}`.
Alternatively to load only job 3:

```
job = kitLoadJob(jobset,3)
```

Setting up a job

To set up a new job, you will need to produce a **jobset** file, for this you will use the `kitGUI` function. This jobset information needs to be assigned to a variable in MATLAB so that it can be used later, so type something like:

```
jobset = kitGUI
```

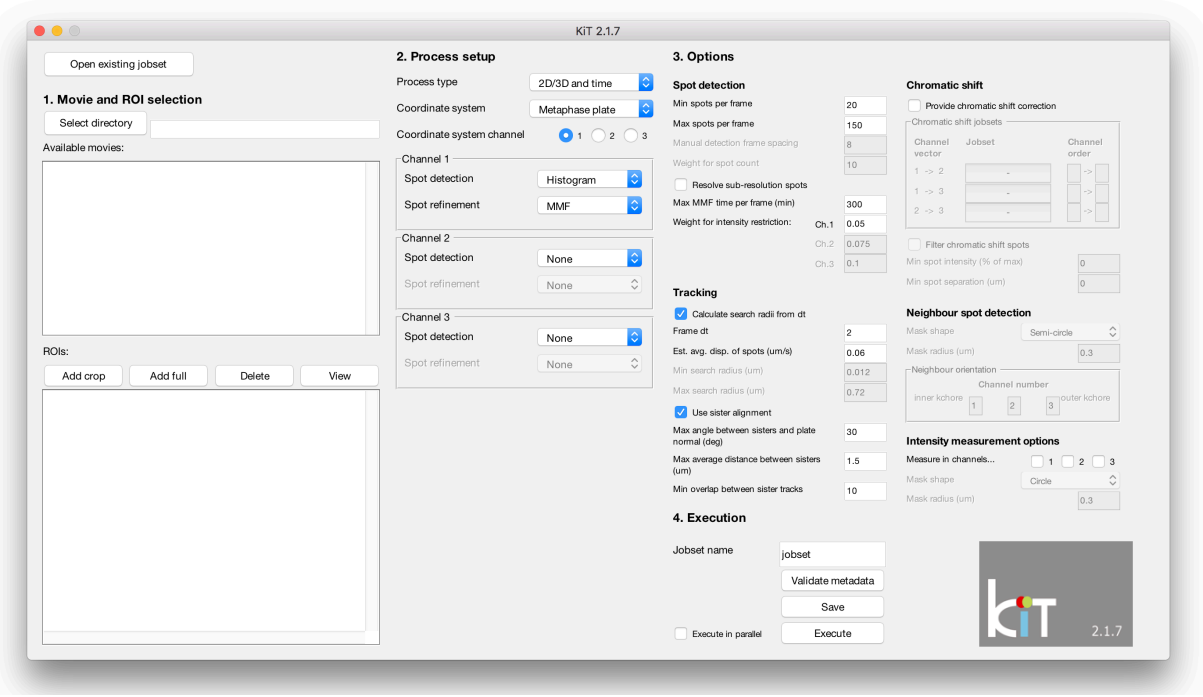
where `jobset` is the variable. When setting up multiple jobsets, you may want to call them something more specific to each experiment.

When running this command, a panel will appear which contains all the necessary components to get you started on setting up jobs (Figure 1).

Open existing jobset

If a jobset has been created previously, it is possible to re-open it if any changes need to be made. You may want to use an old jobset as a template for a new one, which can be done by simply changing the home folder and filenames.

Figure 1. Setting up a jobset.



1. Movie and ROI selection

Here the user will choose the folder containing all movies, and to select which movies within this folder will be tracked by defining a region of interest (ROI) for each.

Select directory

Clicking this button asks the user to select a single home folder containing all the movies wanting tracking. The jobset file will be saved in this folder.
Movies can be stored within their own folders within this home folder – KiT will still locate them. Note that once a jobset file has been created, any changes to movie names or folder names within the home folder will cause the jobset to crash when running at a later date.
Once a directory has been selected, a list of files contained within the folder and all sub-folders will be shown in the panel below (Figure 2).

ROIs

In order to select specific movies for tracking from the full list of files, highlight them (Figure 3) and designate a region of interest (ROI). **Only movies with a specified ROI will be tracked.**
ROIs either surround the entire image xy-plane (by pressing *Add full*), or can be limited to a cropped area in xy (using *Add crop*). The latter may be helpful to speed up processing and/or to remove unwanted fluorescent signal outside the cell.

When cropping, each movie will be shown in turn (z-projected, and t-projected for 10 equally-separated time points; Figure 4). Select *Add ROI*, then drag a box around the selected region. Once happy with the selection of region, double click inside the box. The box will turn yellow, and the button labelled *Finish* will become available: click *Finish*.

Note that ROIs can be provided for each movie individually, allowing for only some movies in a jobset to be cropped, the remaining using the full xy-plane.
Once ROIs have been selected, they will appear in the panel below (Figure 5).

Highlighting a ROI and selecting *View* allows the user to view previously designated ROIs. Similarly, instead selecting *Delete* allows the user to delete the highlighted ROI – remember that this will also remove the movie from the list of movies being tracked.

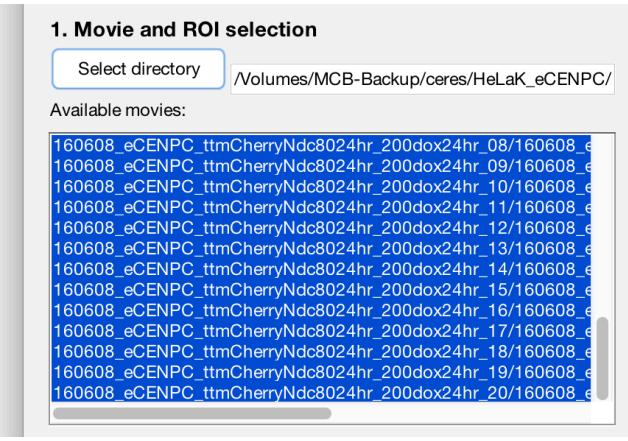
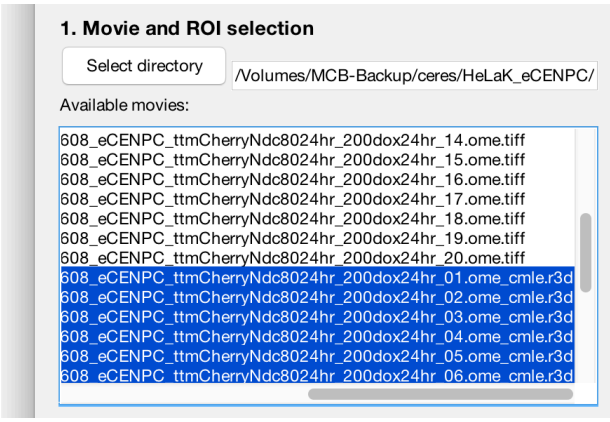


Figure 2. Select directory
Movie files are listed within the 'Available movies:' panel. Any files compatible with bio-formats will be shown here.

Figure 3. Selecting movies for tracking
Highlight the movies for tracking, and proceed with ROI selection. Here, only the .r3d format movies are being selected, as they are the deconvolved versions of the .ome.tiffs.



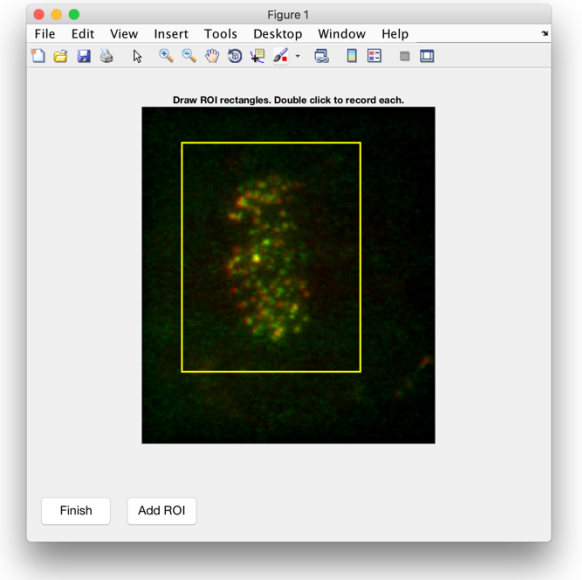
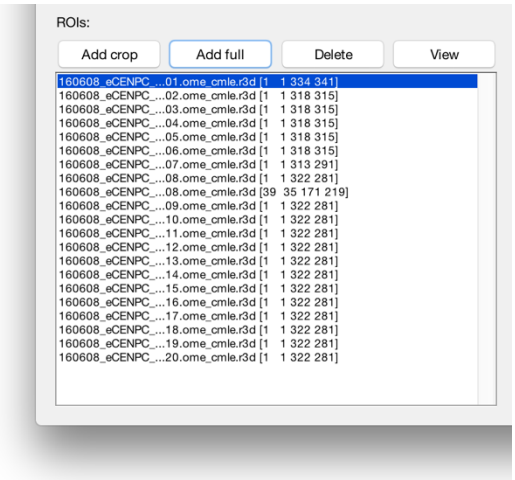


Figure 4. ROI selection.
Each cell will be shown in turn. Select *Add ROI*, draw a box around the cell, then double click within the box. The box should turn yellow as shown. If multiple ROIs are to be added, click *Add ROI* again and draw a second box. Click *Finish* once all ROIs are selected. If instead no crop is necessary, simply click *Finish* without adding a ROI.

Figure 5. Panel of selected ROIs.
All selected ROIs are shown in this panel. Line formatting is as follows:
filename [first_x_coord first_y_coord cropSize_x cropSize_y]
Selecting an individual ROI allows for them to be viewed or deleted.



2. Process setup

Here the process used to run the tracking will be defined, including channel-specific spot detection and refinement methods to optimise the output.

Process type

There are currently three processes of which KiT is capable (Figure 6):

- *2D/3D + time* for time-course movies
- *2D/3D* for single time point images, and
- *Chromatic shift* for images from which to calculate chromatic shift.

As per their labels, each case also accepts single z-plane movies/images. Select the one most appropriate for your movie data.

Coordinate system

Ultimately, KiT was developed to detect fluorescently-labelled kinetochore proteins during prometaphase and metaphase, times in mitosis during which the kinetochores are predominantly, if not all, located in a plate-like structure at the mitotic spindle equator. In KiT, the coordinates of these fluorescent spots can be defined in one of two coordinate systems (Figure 7).

The image's xyz-axes. The origin of the coordinate system is at the centre of mass of all spots detected, while the direction of each the x-, y- and z-axes are equivalent to the direction of the image's x-, y- and z-axes. This can be selected by choosing *Centre of mass* from the drop down menu.

The metaphase plate. The origin of the coordinate system is at the centre of the metaphase plate. The x-axis points normal to the plane that describes the metaphase plate, while the y- and z-axes describe orthogonal axes in the plane. The plane representing the metaphase plate can be calculated in one of two ways:

- Calculating the eigenvectors of the distribution of kinetochore coordinates detected in 3D, and using these to define a 2D plane representing the metaphase plate. For this you need to select *Metaphase plate*.

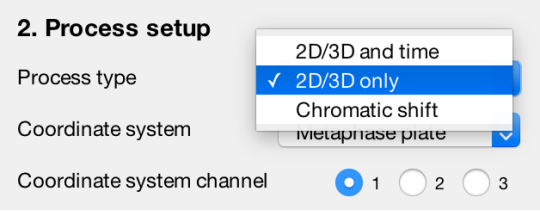


Figure 6. List of options for process type.

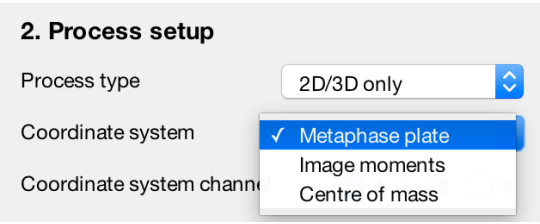


Figure 7. List of options for coordinate system.

- Calculating image moments based on kinetochore intensities. For this you need to select *Image moments*. This is helpful when there is limited, or no, z-directional information in which to fit a metaphase plate.

Coordinate system channel

The coordinate system channel defines the channel used to process calculation of the coordinate system described above. Typically, this should be the channel most likely to produce the best-resolution spot localisation.

Spot detection

Within this drop down menu, there are a list of methods by which KiT will either automatically or semi-automatically locate fluorescent spots. When a given channel does not need to be processed for spot tracking, *None* should be selected. Otherwise, there are five options for spot detection (Figure 8).

Histogram. The brightest pixels in an image are localised by analysing the tail of intensity histograms, either using absolute intensities, dark noise, or poisson noise.

Adaptive. An adaptive version of the histogram method.

Wavelet. Details on this will be provided soon.

Manual. For movies with only a small number of spots ($n \leq 20$), for example when attempting to track spindle poles or specific pairs of kinetochores alone, the user can specifically select spots every n timepoints. Gaps between these time points are then filled by searching in the vicinity of the spots provided.

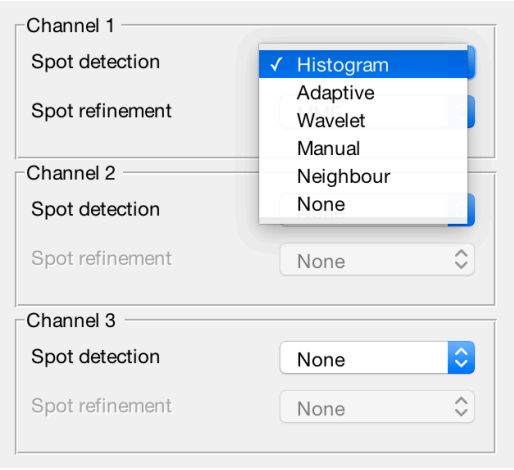


Figure 8. List of options for spot detection.
Spot detection is selected for each channel. If no spot detection is required, select *None*.

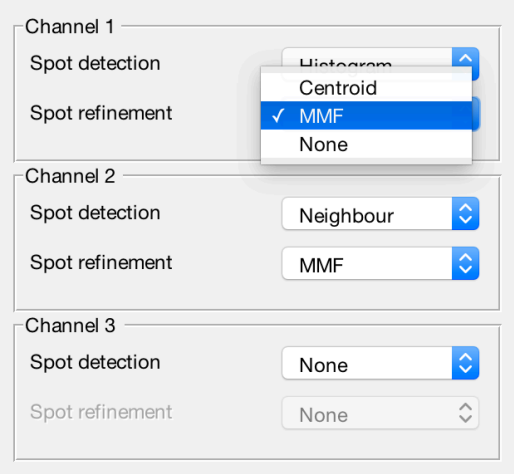


Figure 9. List of options for spot centre refinement. Spot refinement is selected for each channel to locate sub-pixel approximation of each spot centre. *None* can be selected to provide only full pixel coordinates.

Neighbour. This spot detection method can only be used for channels **not chosen** as the coordinate system channel. For the given channel, spots are detected within a mask in the vicinity of the spots detected in the coordinate system channel. This method assumes that for every spot in the coordinate system channel, there is at most one spot in the neighbour detection channel. This is helpful for channels with spots of low signal:noise ratio (SNR), which may not be easily detected by other spot detection methods.

Spot refinement

Once spots have been detected, their centres can be more accurately measured. If no refinement is required, then *None* should be selected. Otherwise, this can be done by one of two methods (Figure 9).

Centroid fitting. The centroid of a spot is defined as the centre of mass of a spot's pixel intensities.

Mixture model fitting. This method fits 3D Gaussians to each spot, however also allows for multiple Gaussians to be fit when spots are localised close to one another. This is especially important within a packed metaphase plate. This is particularly important when analysing high temporal resolution dynamics, and when measuring sub-pixel distances.

3. Options

Some key options can be changed during job setup. These are divided into groups.

Spot detection & refinement

These options refer to either spot detection or refinement parameters (Figure 10).

Min and **max spots per frame**. The histogram cutting algorithm maximises the number of spots detected, aiming to find a number of spots within this range. If all three methods of histogram cutting fail to find a numbers of spots within this range, the closest number will be taken.

Manual detection frame spacing. The number of time points between adjacent manual detection time points. Reducing this number allows for more precise spot detection.

Weight for spot count. This parameter affects how strictly the adaptive histogram cutting will adapt. The larger this is, the more adaptive the cutting. Recommended range is [1 10].

Resolve sub-resolution spots. Ticking this will allow the mixture model fitting (MMF) to attempt to detect undetected spots that may be within such close proximity to already-detected spots that their signals are interfering with one another. This will improve spot refinement in crowded metaphase plates, however significantly increases tracking time.

Max MMF time per frame. The maximum amount of time for running MMF for a single time point. If a single time point exceeds this time, tracking will cease for this movie. If no limit is to be imposed, this should be set to 0.

Weight for intensity restriction. This is a per-channel parameter to determine the significance of the intensity of each individual spot during MMF. They take a value between 0 and 1, and the larger this value, the more lenient the restriction.

Figure 10. Spot detection & refinement options. Shown are the default options for detection of kinetochores in movies where channels 1 to 3 are wavelengths 488, 561 and 647, respectively.

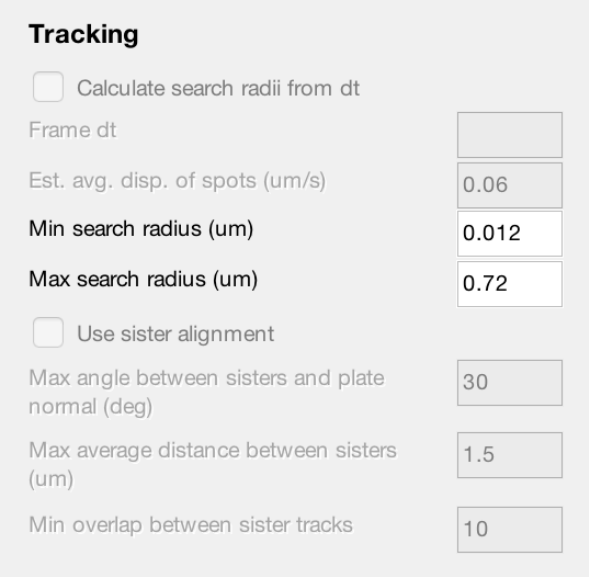


Figure 11. Tracking options.

Tracking

These options refer to tracking parameters (Figure 11).

Calculating search radii. The minimum and maximum search radii are the range within which spots in adjacent time points need to be separated for them to be considered the same spot. These are either defined manually in µm using **Min search radius** and **Max search radius**, or can be calculated by providing the time lapse (**Frame dt**) and the estimated average displacement of spots (**Est. avg. disp. of spots**).

Use sister alignment. Ticking this will mean the sister kinetochore pairing algorithm ensures that the average angle between a candidate pair of sisters and the normal to the metaphase plate (i.e. twist) is within a certain number, defined by **Max angle between sisters and plate normal**.

Max average distance between sisters. Also used by the sister kinetochore pairing algorithm, this is the maximum average distance between a candidate pair of sisters.

Min overlap between sister tracks. The minimum number of time points over which two tracks need to be overlapping for them to be considered a valid sister pair.

Chromatic shift

These options refer to chromatic shift correction parameters (Figure 12).

Provide chromatic shift correction. Ticking this will allow for jobsets to be provided to define chromatic shift between given pairs of channels, labelled as Channel vector in the first column of the table.

Jobset. For each Channel vector, selecting the button allows the user to select the jobset for a previously-run job to calculate chromatic shift between those channels, known as the chromatic shift jobset.

Channel order. This defines the channels within the chromatic shift jobset that represent the channels in the Channel vector.
For example, the jobset being created contains three channels: 488 , 561 and 647 nm, in that order. The chromatic shift from wavelength 488 nm to 647 nm is to be provided, i.e. for Channel vector 1 -> 3. Prior to jobset setup, a chromatic shift jobset was run imaging only 488 nm and 647 nm wavelengths in that order to calculate the chromatic shift, and so the vector within the chromatic shift jobset is 1-> 2. The channel order defined here would then be 1 -> 2, as this is the equivalent Channel vector within the chromatic shift jobset for use in the new jobset being created.

Filter chromatic shift spots. Ticking this will allow chromatic shift spots to be filtered using the following parameters:

Min spot intensity. The minimum spot intensity of spots within the chromatic shift jobset compared to the maximum spot intensity within each image. The maximum is in fact defined as the mean of the 20 brightest spots in the image, in order to avoid a single bright spot removing more spots than necessary.

Min spot separation. The minimum distance between spots. If two or more spots are closer together than this distance, they will be omitted from chromatic shift calculation.

Neighbour spot detection

These options refer to neighbour spot detection parameters (Figure 13).

Mask shape. This defines the shape of the mask within which a neighbour channel is detected in the vicinity of the coordinate system channel. The *circle* option is direction-independent, however *semi-circle* and *cone* require a metaphase plate fit, as they are directed towards the inner or outer kinetochore, depending on the marker orientation. If no plate fit was found, neighbour detection defaults to *circle*.

Mask radius. The radius of the mask.

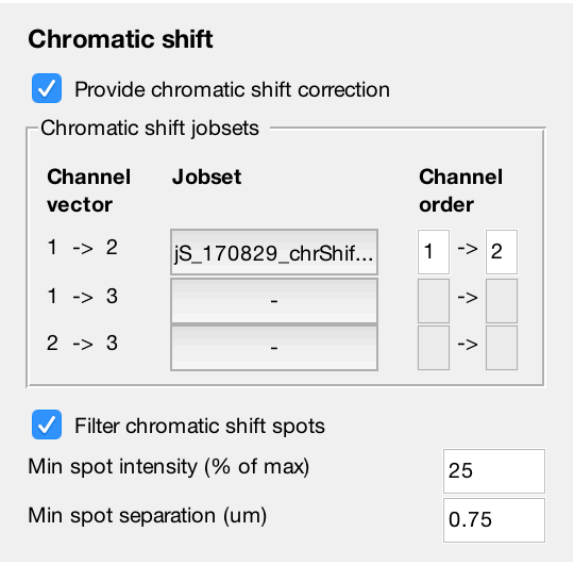


Figure 12. Chromatic shift options.
Shown are the representative options for a chromatic shift correction being provided between channels 1 and 2, where spots for chromatic shift calculation are minimum 25% the maximum intensity, and at least 750 nm away from another spot.

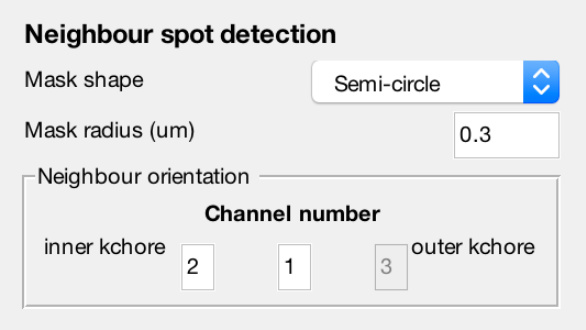


Figure 13. Neighbour spot detection options.

Neighbour orientation. The orientation of kinetochore markers, defined by their channel number.
In the example in Figure 13, channel 2 is the inner-most kinetochore marker, with channel 1 being the outer-most. If a third channel was imaged, in this case it would have instead been the outer-most, with channel 1 being between channels 2 and 3.

Intensity measurements

These options refer to intensity measurement parameters (Figure 14).

Measure in channels... Each channel ticked, intensity measurements will be made. For those channels where spots have been detected and refined, the intensity measurements will be made around the refined spot centres. For those channels where spots have not been detected, intensity measurements will be made relative to the coordinate system channel. This can be particularly useful when measuring intensities of structures which are either not spot-like, e.g. microtubules or crescents, or are too dim to reliably measure a spot centre, e.g. Mad2 in late metaphase.

Mask shape. This defines the shape of the mask within which intensity measurements are made. The *circle* option is direction-independent, however *semi-circle* and *cone* require a metaphase plate fit, as they are directed towards the inner or outer kinetochore, depending on the marker orientation. If no plate fit was found, intensity measurement defaults to *circle*.

Mask radius. The radius of the mask.

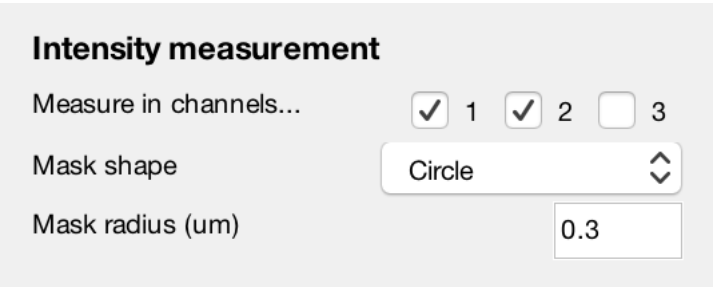


Figure 14. Intensity measurement options.

4. Execution

Jobset name

Provide the name of the jobset file here. Make this specific to the experiment and condition.

Validate metadata

A separate panel will open to allow the user to check through the metadata of each individual image/movie for which a ROI was selected (Figure 15). For each file, the filename is printed at the top of the panel, and each of the following metadata read:

- Frame size: [x, y]
- Number of *z*-slices
- Number of time points
- Number of channels
- Wavelength in each channel
- Pixel size in [x, y, z]
- Time lapse
- Numerical aperture of microscope objective

For each of these parameters, except for Frame size, metadata can be changed. If metadata is consistent throughout an entire experiment for **every** parameter, it is possible to tick *Apply to all?*, which will speed up validation. While validation is not required, it will both ensure that metadata is correct, and speed up the process of opening movies throughout tracking and analysis. Once completed, there remains the option to *Re-validate*.

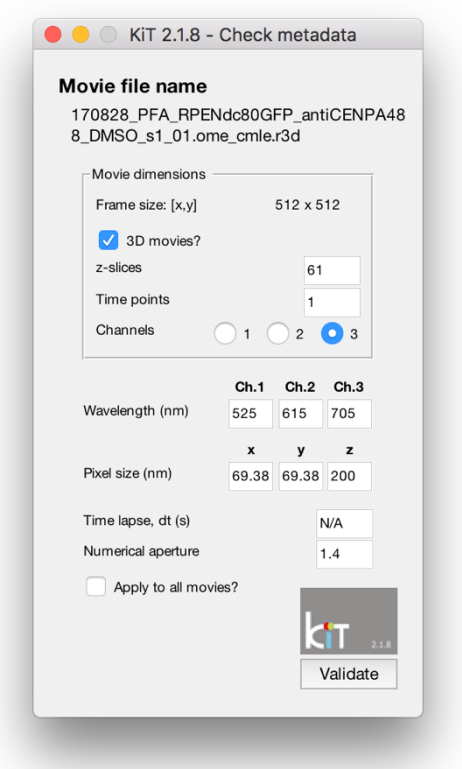


Figure 15. Validation of metadata.

Save

By saving the jobset, it will be possible to run the jobset manually once the GUI has closed. If multiple jobsets are to be run at once, they can each be saved using this method and run later using a single command.

Execute

Alternatively, the jobset can be run immediately by selecting *Execute*. If your version of MATLAB is set up to run parallel processing, *Execute in parallel* can be ticked to speed up tracking.

Analysis Tools

Here will comprise details of a number of analysis tools for running after tracking is complete.

Submitting Bug Reports

If any errors occur during tracking or while running analysis packages, please submit a completed version of the report in Appendix A.
Please send an e-mail to *chrissmith.software@gmail.com* containing this form, including all supplementary files. If any files are too large for e-mail, Dropbox and Google Drive requests are encouraged.
Describe the problem in as much detail as possible, as this will aid in more quickly resolving the issue. Screenshots are encouraged if possible. In order to design and confirm a solution, the error will need to be reproduced. Please provide all files required to run the process, and if necessary provide some information about any commands you ran prior to encountering the problem. Please also feel free to make suggestions for reasons for the issue.

Appendix A: Software Bug Fix Request Form

Name:	
E-mail address:	
Date (dd/mm/yy):	
Software package:	
Software version:	
MATLAB version:	
Description:	
Error message (if applicable):	

List of files provided:

Guidance for error reproduction:

Suggested solutions/extra information: