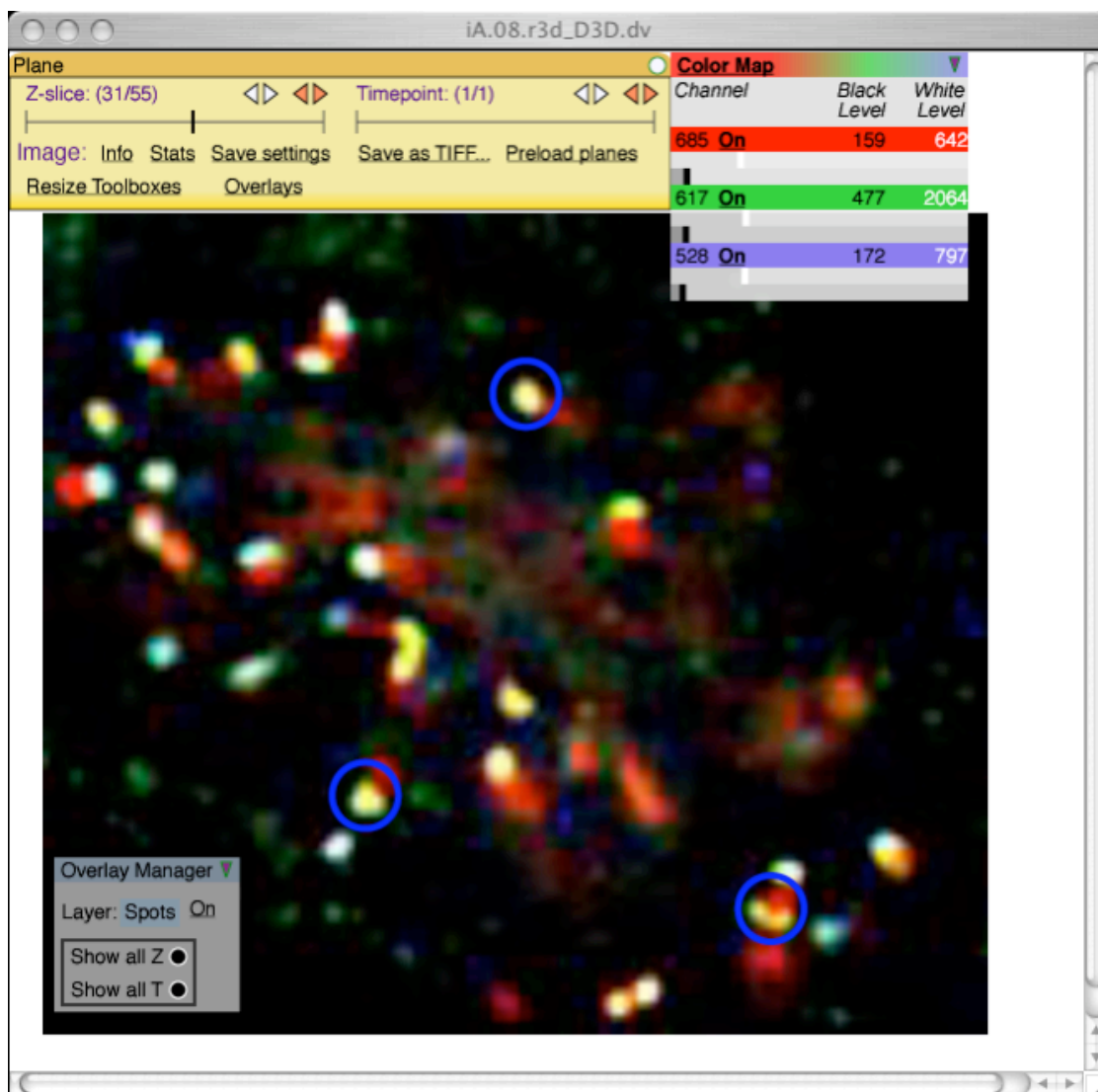


FindSpots (OME v2.5.1) user guide



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The small print...

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1. Introduction

FindSpots is a module within the Open Microscopy Environment (<http://www.openmicroscopy.org>) designed for the detection and analysis of objects within microscope images, in particular fluorescence microscopy images.

The aim of this document is to provide guidance about installing and using FindSpots. In the following sections, we describe how to:

- prepare images for analysis in FindSpots
- set up and run a FindSpots analysis
- import the data into Microsoft Excel for further analysis

NB: Installation of OME and access to the OME server – Please note that this is explained elsewhere: see the OME installation user guide at <http://www.openmicroscopy.org/howto/index.html> and the README file accompanying the Macintosh OME installer (see <http://www.openmicroscopy.org/install/mac-osx.html>).

Note: As an example of the issues to be considered, we describe here how to apply FindSpots for the analysis of kinetochore proteins in images of kinetochore-labelled cells. However, the general points made are applicable to other types of image as well.

An online 'getting started' tutorial and overview of OME can be found at <http://www.openmicroscopy.org/getting-started/>

2. Image formats

Any image file imported into OME can be analyzed with FindSpots. OME is able to import a large number of file formats. Currently supported formats can be found at <http://www.openmicroscopy.org/getting-started/import.html>.

FindSpots can be used not only for fluorescence microscopy images, but also for electron microscopy images and any other microscope images that can be saved in a suitable file format. Indeed, FindSpots & OME are not limited to microscope images, and can also be used to store, manage and analyze images from other types of equipment.

3. At the microscope: image-collection advice

When collecting images, it is important to consider how the image will need to be processed (e.g. by cropping) and how it will be analysed, as taking this into account at the image-collection stage, can simplify further analysis.

Ensure that all images from a single 'experiment' to be compared directly against each other (which

typically will subsequently be imported into a single, or perhaps more than one, OME 'dataset' – explained later on), are collected using identical exposure times per channel (i.e. for any one channel, the exposure time is the same for all images), and identical binning settings on the microscope, where this is an option¹.

NB: When capturing images of cultured cells, if possible ensure that cells are not too closely packed next to each other or on top of each other; this will make subsequent image cropping simpler, especially if the software package used for cropping only allows one to specify *rectangular* regions to crop.

4. Pre-processing: preparing images for analysis in FindSpots

Images may need to be cropped before analysis, i.e. some regions of the image may need to be removed (through all optical sections), e.g. to remove unwanted neighbouring cells; and some optical sections may need to be removed, e.g. to remove additional underlying cells. It is likely that this step can be accomplished using the software supplied with the microscope system – for example, in the case of Applied Precision's DeltaVision microscopy system, one can use their softWoRx software (running on Linux): this is a software package principally for image deconvolution of DeltaVision files and for the analysis of DeltaVision files and other image formats. Alternatively, one can use stand-alone image processing/analysis packages as appropriate, for example softWoRx Explorer (a Java application, also by Applied Precision, Inc., for Mac or Windows, <http://www.api.com/lifescience/softworxexplorer.html>); or ImageJ, a freely available package for image processing and analysis (<http://rsb.info.nih.gov/ij>), for which many plugins with advanced features are available.

When dealing with deconvolved files, a simple but helpful step is to prepare first, a 'before cropping' directory, containing sub-directories with just the 'original' deconvolved images for any one experiment (i.e. before any cropping is performed); this will make it easier to select the right ones to crop, instead of manually scrolling through large directories which contain also the non-deconvolved images too as well as log files (and minimises the risk of opening and working on a non-deconvolved image by mistake).

It is also recommended to prepare a separate 'after cropping' directory, with similarly named sub-directories as for the 'before cropping' sub-directories. Cropped/edited files will be saved in here. This has a number of advantages. First, it allows one to go back to the original file if one needs to adjust the cropping. Second, it is often helpful to look back at the original files to see the full 'context' of a closely cropped image, e.g. to help confirm the cell-cycle stage.

Crop the images as needed, to produce images that do not have too much non-cell 'black space' around the cells, and which avoid including kinetochores from neighbouring/overlying cells. Because software is often limited to rectangular shapes for the cropping, it may be necessary to compromise and miss out some kinetochores from the desired cell, in order to avoid simultaneously including kinetochores from neighbouring cells.

NB: it is best to scan through all the Z-sections of each image, because a file can appear to contain only one cell, but the other cell(s) may only be visible in a sub-set of the Z-sections.

¹ Note that it is okay to collect different numbers of slices in different images; or to vary the XY size of the images collected (and images will likely all be cropped somewhat anyway). Ideally the Z-sections should extend above and below the region of interest (here, the kinetochore-containing region), by about the same amount for each image (since this could skew the overall mean pixel value, which may be used to determine an automatic threshold value for that image) but in practice this is not critical.

Try to avoid images that contain large 'blotches' of signal, e.g. due to cell debris or precipitation of fluorophore-containing material – or where possible crop these areas out or avoid those Z-sections. Otherwise, note which files this occurs in, as it may be necessary to alter the method used for determining background in such images².

Re-save them with the same filename as originally, but in the corresponding sub-directory in the 'after cropping' directory.

5. Connecting to OME

This and subsequent steps will require use of a web browser. For more details about how to access OME using a web browser, see the OME installation instructions, available from the OME website.

Note that if using a Mac, it is recommended to use Apple's 'Safari' web browser (available as standard in Mac OS X); for Windows, Internet Explorer is recommended.

Connect to an OME server and log in.

6. Importing images into OME

Before any analysis can be performed on the images, they must be imported into OME.

Create datasets

A fundamental unit within OME is the 'dataset'. This is a group of one or more still images or movies. Analyses such as FindSpots can be performed on whole datasets at a time, so it is generally convenient to place all images to be directly compared against one another, in a single dataset.

Datasets can only contain a single copy of any one image that has been imported, but images *can* be listed in more than one dataset (see 'Add Images' later).

Datasets can be created in advance of image import, by clicking on 'Dataset' under the 'Create' menu (the list of menus is displayed on the left-hand-side of the screen). However, you may find it simpler to create datasets as needed, during import.

To import images, click on 'Images' under the 'Import' menu. One can choose whether to import files into an existing dataset or a new dataset.

To import files into an existing dataset, click the 'Existing dataset' button, then select the dataset name from the pop-up menu adjacent to it.

Alternatively, to create a new dataset, click the 'New dataset with name' button, and enter a name for the

² Whereas it is often possible, if the spots are small enough compared with the image as a whole, to estimate 'background' using the mean of the whole image, this may not be valid if there are large 'blotches' or inhomogeneities of colour in the image, in which case the mean should be estimated from manually selected 'normal' regions of the image.

dataset in the box to the right, together with a more detailed description (if desired) in the box below. When choosing the dataset name, avoid commas, ampersands (&) or symbol marks (e.g. + etc), as this can cause problems when importing the FindSpots data subsequently into Microsoft Excel.

If these have already been used in the dataset name, try to rename the dataset within OME – this can be done by editing the name and clicking 'Save' (unless the dataset has become 'locked' by OME, in which case one should contact the local OME database administrator, or the OME developers).

Selecting files to import

NB: Be careful to select the right images to import into the chosen dataset, as it is not readily possible to delete images once imported though they can be removed from datasets, and/or reassigned to different datasets later on³. One can *add* images to datasets later on using OME's 'Add Images' and 'Remove Images' commands to add or remove *already imported* images into that dataset – see below.

To find the files to import, use the 'Directory Listing' window in the lower portion of the page. Click on directory names to navigate to the directory where images for import are stored. Once a list of images is displayed, choose the files to import: either click on 'select all' to select all files in the current directory, or check the boxes next to the files to be imported.

Once the files have been selected, click on 'Add to queue', which will cause these files to be added to the queue on the right-hand panel titled 'Import Queue'. Files can be removed from this list prior to starting the import, by selecting the check boxes next to them, and clicking 'Remove from Queue'. Files can be added to the import queue from different parts of the directory structure.

Once you are satisfied that the correct files have been chosen for import, and the correct dataset has been selected, click on 'Import files', and file import will begin.

To check progress, click on 'Tasks' (under 'Options', at the bottom of the menu).

Note that it is not possible to import a file that has already been imported into the current OME installation, even if the filename or location is changed. This is because a 'digital signature' is created for each file based on its contents. This is a feature designed to maintain database integrity: otherwise, the same file may in practice be imported multiple times, thereby losing the connection between all the actions performed on what is actually the same image. It also saves storage space.

Instead, to add a file that has already been imported, one should instead use the 'Add images' command to add the image to a new or previously created dataset (described below). Alternatively, one can slightly modify the image (e.g. slightly crop it), which will alter its corresponding digital signature, and allow a copy of it to be imported.⁴

³ This was chosen as it has benefits from a design and database-integrity point of view.

⁴ OME can also be run via the Unix command-line, and it is possible to use this to import a file more than once. This more advanced method of interacting with OME is beyond the scope of this manual. Please contact the developers for more information on this.

7. Choosing a dataset

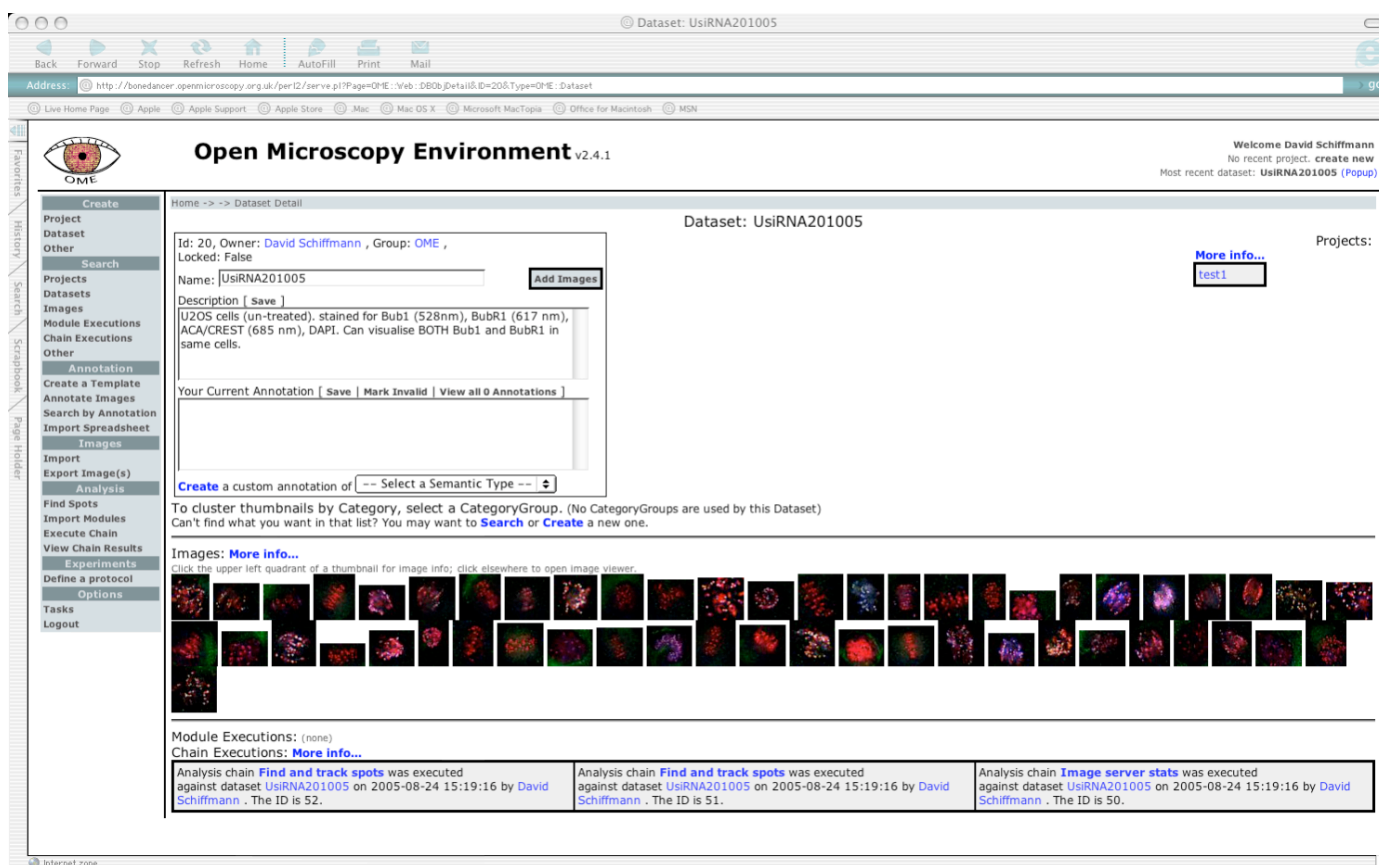
If one has created more than one dataset, it may be necessary to switch between these. Indeed, before running FindSpots, it is necessary to first open the dataset on which FindSpots is to be run.

OME home page after logging in. At any time (at the homepage or elsewhere), clicking on 'Dataset' under the 'Search' heading will allow you to choose a dataset to process.

To do this, first click on 'dataset' under the 'Search' menu and you will be presented with a view as shown below, containing a few images from each dataset.

Clicking on 'Dataset' under the 'Search' menu shows this view, with thumbnail illustrations of a few images from each dataset.

Click on the name of a dataset to proceed to a detailed view of the chosen dataset. You will be presented with a view like this:



Summary and description of the selected dataset. At this point, one can start a FindSpots run by clicking on 'Find Spots' under the 'Analysis' menu, or one can click on 'More info' for more information about each image, including results from previous FindSpots runs. An alternative way to reach the image detail page is to click on the upper-left corner of an image thumbnail displayed in any OME page. Clicking anywhere else on the thumbnail will launch the image viewer for that image.

8. 'Pre-flight' quality control

Before running FindSpots, it is recommended to check what files have been imported.

Ensure all files have been imported as intended

NB: After importing images, but before running FindSpots, it is recommended to compare what is in the actual OME dataset with the list of pre-processed files that one *intended* to include in there. Check if any were omitted from the import. This is particularly important if one intends to import the resulting data into Microsoft Excel. Because of the way the final output is generated in Excel by the import macro, it is much simpler to run the FindSpots analysis and the subsequent import into Excel, with *all* the relevant images files in place on the OME dataset, rather than add and process them in stages later on.

Using the 'Add images' button

Images that have *already* been imported into OME, can be added to a dataset using the 'Add images'

command (shown in a later screen-shot). The 'Add Images' and 'Remove Images' buttons are located next to the dataset name, and is an option only available in the dataset detail page – which one obtains by clicking on a dataset name from any other page (such as a search page).

'Add images' has several potential uses.

If one would like to add certain images to a dataset which have already imported into *another* OME dataset, one can use the 'Add images' button to add these to the dataset being viewed without having to re-import them. Indeed, as mentioned above, it will not actually be possible to re-import images if they have already been imported into OME. Any images added in this way continue to also be in the other dataset they were in unless they are removed from it. Similarly, images removed from datasets using the "Remove Images" command continue to exist in other datasets or "unattached" to a dataset. Currently, it is only possible to fully delete images from an OME system using OME's command-line administration tools which are beyond the scope of this document (see <http://www.openmicroscopy.org/system-admin/>).

Or, if one wants to run an analysis on just a sub-set of the images that one has already imported into OME, one can create a new dataset and use 'Add Images' to add only the desired images to this new dataset (these images can come from any number of different datasets). This can be useful, for example, to save time by only analysing a few images whilst trying out different FindSpots parameters, or because one is in fact only interested in a sub-set of them, perhaps because one has imported some 'unwanted' files by mistake.

9. Using the viewer

A large part of the interaction with OME/FindSpots is via the web-based OME viewer. Images displayed via the web interface of OME are generated using a plug-in called Adobe SVG viewer⁵, which needs to be installed in order for images to be displayed. If this is not already installed, a message will appear explaining this, with a link to download it.

The viewer has a number of 'floating panels' to control the display. The largest, on the top-left, has sliding controls enabling one to move through the different Z-sections and through time (if the image consist of a time series), either one at a time (white arrows), or automatically (shaded arrows). Note that before using the shaded arrows, it is helpful to click on 'preload panes' – this pre-loads the data for the various Z-sections or timepoints, yielding a smoother transition between them.

There are a number of other panels as well: 'Info' opens up a page in one's web browser, with links to further information about this image, e.g. the different FindSpots analyses performed on it. 'Stats' displays basic statistics, useful for example when establishing parameters for automated thresholding (see below).

The 'Color map' floating panel allows one to change the black level and white level of the image, to switch wavelength channels on and off in the image, and to choose which emission wavelengths to display.

Note that it is possible to zoom in and out when viewing an image. If you're using the Adobe SVG viewer for the Mac with the Safari web browser (the recommended browser to use when using a Macintosh), then

⁵ For more information on this tool, see <http://www.adobe.com/svg/main.html>

apple-click will do a zoom-in, apple-shift-click will do a zoom out, and option-drag will allow you to move the image window around. The Adobe SVG plug-in for Windows works slightly differently (see <http://discover.nci.nih.gov/mim/SVGHelp.html>).

The SVG viewer is limited in that it only allows you to look at 3 wavelengths at a time, because each wavelength is assigned to one of the RGB (red, green, blue) channels. However you can click on the wavelength's label (usually the nominal emission wavelength, sometimes the fluorophore) in the color map, and you will get a popup menu of all wavelengths collected, so that you can assign a different wavelength to one of the RGB channels. This can be seen in the screen-shot later, showing a graphical overlay of FindSpots results.

The Java-based viewer in Shoola (a Java-based interface to OME, which does not yet support FindSpots) is able to blend the four wavelengths in more ways so that you can see them all at once.

10. Setting the spot-finding parameters - theory

In order to use FindSpots to detect and measure objects within an image, it is necessary to set a number of parameters. These are all set using the form shown below.

The screenshot shows the Open Microscopy Environment (OME) v2.4.1 web interface. The browser address bar shows the URL: <http://bonedancer.openmicroscopy.org.uk/per12/serve.pl?Page=OME::Web::FindSpots>. The page title is "Open Microscopy Environment v2.4.1". The user is logged in as "David Schiffmann". The "Selected dataset" is "UsiRNA201005". The "Enter parameters for findSpots" form is displayed with the following fields:

Time: From/to	<input checked="" type="radio"/> Beginning <input type="radio"/> Timepoint
	<input checked="" type="radio"/> End <input type="radio"/> Timepoint
Channel	685
ThresholdType	RelativeToGeometricMean
ThresholdValue	3
Min Spot volume	30

Below the form is a "Run FindSpots" button. The left sidebar contains a navigation menu with options like "Create", "Project", "Dataset", "Search", "Projects", "Datasets", "Images", "Module Executions", "Chain Executions", "Other", "Annotation", "Create a Template", "Annotate Images", "Search by Annotation", "Import Spreadsheet", "Images", "Import", "Export Image(s)", "Analysis", "Find Spots", "Import Modules", "Execute Chain", "View Chain Results", "Experiments", "Define a protocol", "Options", "Tasks", and "Logout".

FindSpots parameters window. Set the parameters for the analysis and click 'Run FindSpots'.

FindSpots defines a spot as any contiguous set of pixels above the threshold value, that are larger than a minimum size pre-set by the user prior to the run. An important parameter to set is therefore the threshold above which a pixel can be considered part of a spot.

This can be fixed by the user (the 'Absolute' threshold option within FindSpots) or it can be calculated automatically by FindSpots on an image-by-image basis for each image within the dataset being analysed. FindSpots includes a number of algorithms for calculating such an automatic threshold, and it is critical to choose a method (and associated parameter(s), if relevant) that is suitable for the images being analysed. Note that in any one 'run' of FindSpots, all images will be analysed by the *same* thresholding algorithm. These automatic algorithms are:

RelativeToMean: where threshold = mean of all pixels in stack + n . standard deviation of all pixels in stack ⁶.

RelativeToGeometricMean: where threshold = geometric mean of all pixels in stack + n . standard deviation of all pixels in stack

FindSpots also has implementations of four other algorithms for automatically determining a threshold: Otsu, Kittler, MomentPreservation and Maximum Entropy, but these are generally not of use for typical fluorescence microscope images, and will give far too low a threshold value to be of use for such images.

The parameter, ' n ', above is referred to within FindSpots as 'ThresholdValue', and is only relevant for *RelativeToMean*, *RelativeToGeometricMean* and *Absolute* methods (altering it will have no effect on the other thresholding methods), which are the three methods most likely to be useful for analysing a series of fluorescence images.

The choice between RelativeToMean and RelativeToGeometricMean will depend on the images in question; although RelativeToGeometric Mean is expected to give a more robust measure of threshold, in a recent study of kinetochore-labelled cells, we found that RelativeToGeometricMean did not give satisfactory results, and used RelativeToMean instead.

The concept behind RelativeToMean and RelativeToGeometricMean, is that they provide a simple but effective, *objective* way to determine a threshold that can (at least to some extent) take account of varying background levels for each image (or more correctly, each stack of optical sections). Although 'Absolute' allows one to set an absolute threshold, it is rarely of use because images will typically vary in background level, and it is desirable to vary the threshold on an image-by-image basis to reflect this, rather than using a *fixed* threshold level.

Minimum spot volume

The other parameter which needs to be set is the 'minimum spot volume', measured in pixels. This is important as by setting it sufficiently high it can, in effect, 'filter out' unwanted spots, e.g. those due to random noise or non-specific antibody labelling, from the output. It should of course not be set too high, otherwise spots of interest may also be lost.

In order to choose a value at which to set this parameter, it can be helpful to first run the analysis and examine output with no, or very low, minimum value set. Next, examine some typical images. If too

⁶ Note that for this and the other automatic thresholding methods, the same threshold is used for all Z-sections within a stack, but if the file also contains a time-series, a new threshold (using the same parameters) will be calculated for each point in the time series.

many spots are detected with the current parameters, one can then increase the minimum spot volume and run the analysis again. It can be helpful to obtain the spot reference numbers (see below) of some of the 'true' expected spots, and examine the numerical output to determine their volume, so as to have a better idea of the pixel volume of the expected spots. The 'minimum spot volume' should then be set somewhere below this value, but not too close to it, so as to leave enough room for variation in spot volumes.

11. Setting the spot-finding parameters – in practice

As can be seen from the preceding section, a critical parameter to set for a FindSpots analysis is the threshold – all contiguous pixels whose intensities are above the specified threshold are considered to be part of one spot.

Since the most useful threshold methods will be RelativeToMean and RelativeToGeometricMean, this in effects means that the critical parameter to set is 'ThresholdValue', or n above. The greater n is, the higher the threshold will be, and the fewer and/or smaller the spots will be.

Visualising the effect of threshold

In order to know what to set ThresholdValue to, it can be helpful to visualise the effect of different threshold values on the image prior to running FindSpots. To do this, first open the image in a suitable viewer; although this need not be a viewer within OME, it may be convenient to use one of the OME viewers for this.

To open an image using the standard viewer described above, click on one of the images in dataset to open the viewer: clicking anywhere on the small (thumbnail) view of an image will open the viewer, except for the top-left corner, which will open a page with information about the image and commands that have been run on it.

The aim here is to set the white level and black level to the same value so that you get a binary image (i.e. pixels are fully on or fully off). Displaying the image in greyscale and selecting the proper channel for the greyscale display is one way, but you can do the same thing with the red, green or blue channels.

To set the white level and black level to the same value, set the black level first with the slider so that the spots you want to find are visible, but the background is not. Now click above and to the left of the black level slider position (i.e. in the white-level slider area, to the left of the black-level you set). The white slider will jump and set itself to the value of the black slider. What you should see in the display is a binary image. Keep adjusting the sliders so that the binary image displays only the spots you want found.

The numerical value of the white/black level is a threshold to use for FindSpots. You can use this numeric value directly when applying the 'Absolute' threshold method in FindSpots.

Usually, you will have a sprinkling of pixels that are not in your spot, but are still above the threshold - noise in other words. Since these are normally single pixels or generally, much smaller than the spots you're interested in, the minimum spot volume parameter in FindSpots lets you filter these out (the spot volume is the number of pixels in the spot).

Moving from an 'absolute' threshold to a 'statistical' threshold

As mentioned above, the 'absolute' threshold method is usually not as useful as the 'statistical' methods which automatically calculate the threshold anew for each image. This is because the absolute pixel intensity reported for the white/black level is not very 'robust', i.e. it needs to be varied to account for differences between images, which may be due to differences in background between images, or perhaps photobleaching in different timepoints. A more robust value that is less sensitive to differences in background is therefore required.

For this reason you can specify the threshold as some number of standard deviations (sigmas) above the mean or geometric mean. In the web-based viewer described above, click on the 'Stats' button. This will display means, geometric means, sigmas (standard deviations) and geometric sigmas for all of the channels in the image. To get a more stable threshold value, take the absolute threshold determined earlier through moving the sliders around as described above, and convert that number to some number, n , of sigmas above the mean (or geosigmas above the geomean). i.e. $n = (\text{absolute threshold} - \text{mean}) / \text{sigma}$. Then, use this parameter (it does not have to be a whole number) as the parameter (referred to as 'ThresholdValue') for a statistical threshold, i.e. RelativeToMean or RelativeToGeometricMean.

Worked example:

Let us assume that for a particular image, by adjusting the sliders, a threshold of **1205** seems optimal for the chosen wavelength channel in the image. In the 'Statistics' window, look up the mean and standard deviation (called 'sigma') of the pixel intensity for the image.

Let us assume that the mean is **503** and the standard deviation is **223**.

Calculate how much the threshold is, above the mean: $1205 - 503 = \mathbf{702}$

Calculate how many standard deviations this value (702) corresponds to: $702 / 223 = \mathbf{3.5}$ approx

Hence for this image, the ThresholdValue setting should be 3.5

It is recommended to check a few other typical in the dataset, to ensure that a ThresholdValue of 3.5 will give similar results. To do this, open another image and view the 'Statistics' window for that image; let us assume that the mean is 398 and standard deviation is 189. In that case, using a ThresholdValue of 3.5 will yield a threshold of $398 + (3.5 \times 189) = 1059.5$. Set the sliders to as close to 1059.5 as possible and scan through the optical sections in the image, to ensure that a satisfactory thresholding is achieved. Repeat this for some other images as desired.

12. Starting a FindSpots run

Before running FindSpots, the dataset on which to run the analysis should be selected. To do this, click on 'Datasets' under the 'Search' menu, and click on the required dataset from amongst the list of datasets. This will open a page showing thumbnails (small pictures) of each image in the dataset. Next, run FindSpots: click on 'Find Spots', under the 'Analysis' menu.

Enter the threshold and minimum spot volume, set as discussed above.

It is also necessary to specify which emission wavelength will be used to define the spots. Note that FindSpots will use the spots defined by this one emission wavelength, to calculate the spot intensity within those spots, not only for the wavelength chosen, but for all the *other* wavelengths in the image as well. This means that one can define spots using a high-contrast label, and then work out the total intensity of some other channel(s) within those spots, even if those other channel(s) do not have such a high signal-to-noise ratio (i.e. even if one would not easily be able to define the spots using those signals alone).

After checking that the parameters have been entered correctly, click 'Run FindSpots'. Immediately after this, one can repeat this to queue further analyses tasks on the same dataset, or choose other datasets and queue FindSpots analyses on them. Or one can perform other tasks in OME, such as importing further images.

Note: Do not attempt to add an image to a dataset (by adding one from an existing dataset, or by importing an image(s)) *whilst* FindSpots is running – it will cause unpredictable and potentially serious problems to the software!

Checking progress

At any time, it is possible to check on the progress of an OME task, such as image import or a FindSpots run, by choosing 'Tasks' from the 'Options' menu. The status will display 'FINISHED' when the task has completed, or an error message will be shown in the task was not completed

Tasks

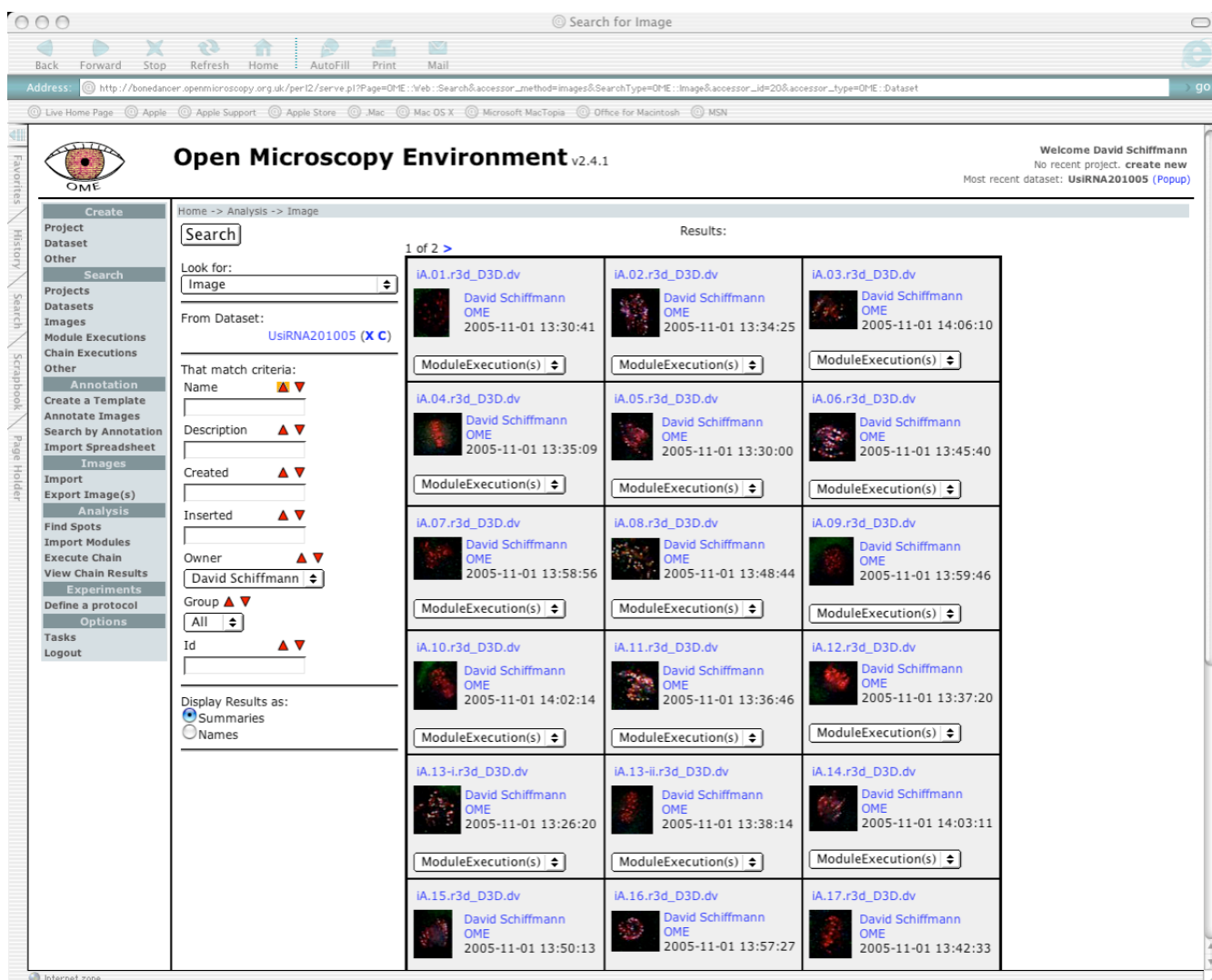
Select	Task	Status	Message	Error	Last step	N steps	Start time	Elapsed time	PID
<input type="checkbox"/>	Executing 'Find and track spots'	FINISHED			129	129	2005-08-30 11:29:18	00:10:22	25670
<input type="checkbox"/>	Executing 'Find and track spots'	FINISHED			285	285	2005-08-30 16:19:24	00:13:12	25667
<input checked="" type="checkbox"/>	Executing 'Image server stats'	FINISHED			22	22	2005-09-02 13:25:20	00:06:35	25669
<input type="checkbox"/>	Importing images	FINISHED	Imported 7 images from 7 files. 7 scanned. 0 unknown format, 0 duplicates, 0 errors.		10	10	2005-09-02 13:09:08	00:22:47	25669
<input type="checkbox"/>	Executing 'Find and track spots'	FINISHED			29	29	2005-09-02 14:55:42	01:53:18	25669
<input type="checkbox"/>	Executing 'Find and track spots'	FINISHED			117	117	2005-09-05 17:54:20	00:06:05	16896
<input type="checkbox"/>	Executing 'Find and track spots'	FINISHED			125	125	2005-09-05 17:56:21	00:08:19	6421
<input type="checkbox"/>	Executing 'Find and track spots'	FINISHED			117	117	2005-09-06 13:53:23	00:05:15	17035
<input type="checkbox"/>	Executing 'Image server stats'	FINISHED			91	91	2005-09-06 14:01:52	00:00:35	6421
<input type="checkbox"/>	Importing images	FINISHED	Imported 1 images from 1 files. 1 scanned. 0 unknown format, 0 duplicates, 0 errors.		4	4	2005-09-06 14:01:04	00:01:23	6421
<input type="checkbox"/>	Executing 'Find and track spots'	FINISHED			121	121	2005-09-06 14:04:09	00:05:29	6418
<input type="checkbox"/>	Executing 'Image server stats'	FINISHED			7	7	2005-09-28 14:20:17	00:00:26	363
<input type="checkbox"/>	Importing images	FINISHED	Imported 2 images from 2 files. 2 scanned. 0 unknown format, 0 duplicates, 0 errors.		5	5	2005-09-28 14:18:58	00:01:45	363
<input type="checkbox"/>	Executing 'Image server stats'	FINISHED			112	112	2005-10-11 15:41:43	00:06:57	12273
<input type="checkbox"/>	Importing images	FINISHED	Imported 37 images from 37 files. 37 scanned. 0 unknown format, 0 duplicates, 0 errors.		40	40	2005-10-11 15:27:24	00:21:16	12273
<input type="checkbox"/>	Executing 'Find and track spots'	FINISHED			149	149	2005-10-11 15:50:59	00:10:42	9597
<input type="checkbox"/>	Executing 'Find and track spots'	FINISHED			149	149	2005-10-11 15:51:26	00:10:06	11999
<input type="checkbox"/>	Executing 'Image server stats'	FINISHED			106	106	2005-10-14 15:25:19	00:12:49	14268
<input type="checkbox"/>	Importing images	FINISHED	Imported 35 images from 35 files. 35 scanned. 0 unknown format, 0 duplicates, 0 errors.		38	38	2005-10-14 14:59:45	00:38:23	14268
<input type="checkbox"/>	Executing 'Find and track spots'	FINISHED			141	141	2005-10-14 15:42:58	00:13:12	11999
<input type="checkbox"/>	Executing 'Find and track spots'	FINISHED			141	141	2005-10-14 15:44:30	00:11:11	14268

'Tasks' window. At any time, one can see a list of current or previous 'tasks' in OME, by clicking on 'Tasks' under the 'Options' menu

13. Inspection of FindSpots results

Visual inspection of overlays

After running the analysis, it is recommended to check some images to make sure the analysis has worked as desired. If the relevant dataset is not currently visible, click on 'Datasets' under 'Search' and then click on 'More info' next to the chosen dataset. A new window will appear.



The view that appears after clicking the 'More info' link for a dataset.

Find the image you wish to open (it may be necessary to move between pages, using the blue > signs at the top of the page), and click on the image title (blue underlined name).

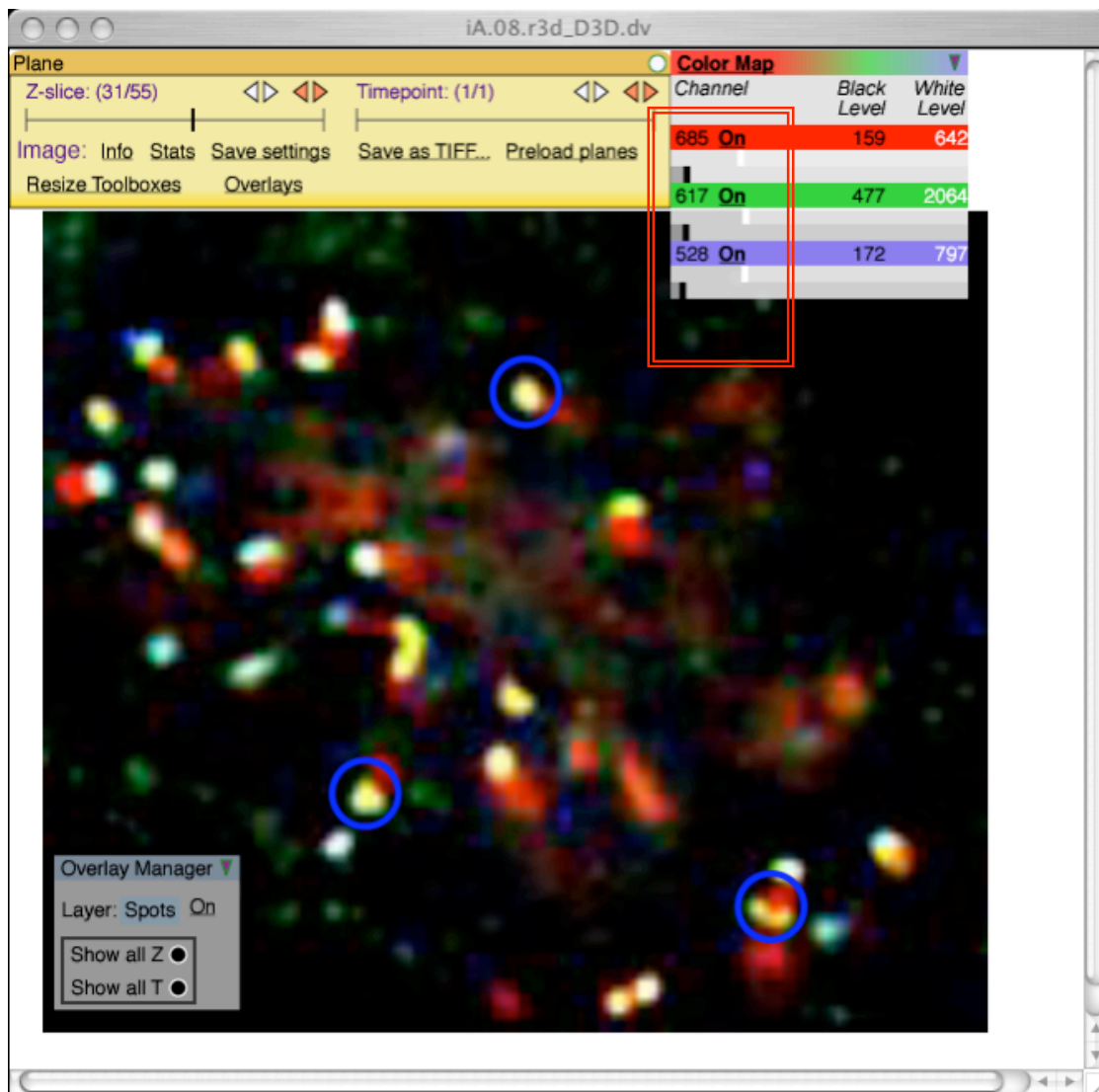
A view like the one below will appear. This is the “Image Detail” view for the selected image. This view can be reached from any thumbnail displayed on any OME page by clicking the upper-left corner of the thumbnail. Clicking on any other portion of the thumbnail will launch the OME image viewer. It is not necessary to click “More Info” in the dataset detail page if the desired image can be recognized from it thumbnail.

Information about an individual image file. This provides access to previous 'Find spots' runs, according to the date/time they were run.

To look up the FindSpots output for this image: on the right under 'More info' will be a number of headings; choose the one called 'Find Spots' (not the one called 'Track spots'). Look for the one nearest the top, which will correspond to the most recent analysis – one can always confirm this by the date/time details provided therein, and by checking that the parameters used for that run, such as ThresholdValue, MinimumSpotVolume or the thresholding algorithm, are as expected.

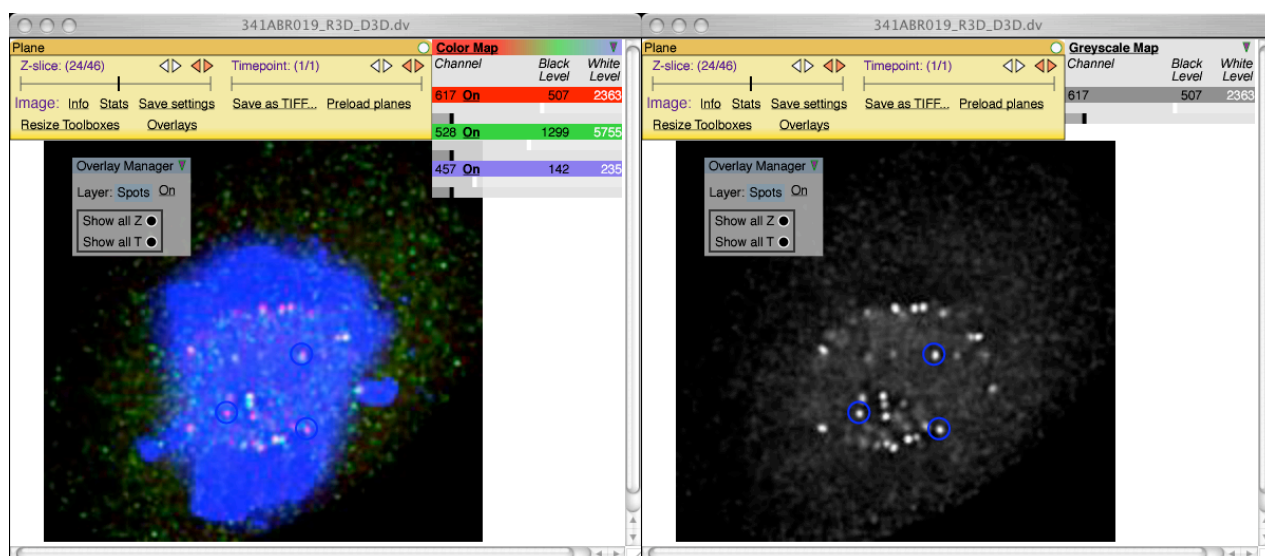
Click on 'View graphic overlay' – this will display the original image. To turn on the display of spots found, click on 'Overlay'.

By default, this will only show spots whose centroids (centers of mass, weighted by intensity of all pixels in the spot) are in the current optical section. However, it may be helpful to simultaneously display spots in *all* optical sections. This can be done by turning on the 'all Z' option: click in the circle next to it, and a white circle will appear to indicate this option is active (one can return to a display of only spots in the current optical section, by clicking 'Show all Z' again). A similar option ('Show all T') allows the user to toggle between a display of spots only in the current time-point, or a display of spots at all times.



Example of FindSpots output overlaid over the original image. One optical section of the image is shown at a time. The blue circles show spots whose centroids are in the particular optical section currently displayed. Note: the above image contains data from four emission channels; however the SVG viewer used here can only display 3 at a time; to change one of the channels to display a different wavelength, click on the wavelength and select the new one from the pop-up menu (see the red boxed area above).

One can toggle between a colour and a greyscale representation by clicking on the 'Color Map' or 'Greyscale Map' headings shown next to the image. One can also turn channels on and off as desired. It can sometimes be easier to change the display mode from colour to grayscale, in order to more clearly see the blue circles outlining each spot that has been found, as shown below.



Changing from a colour to a greyscale display can help to highlight the spots found by FindSpots.

Make sure (by examining a couple of output images with the FindSpots overlay) that the parameters chosen, including the number, n , of standard deviations above the mean, yield output images that show relatively well separated 'spots' (here, kinetochores), i.e. that the actual spots in the image are not all merged into a few very large blotches (which can happen if n is too low), which will give very misleading data. If on the other hand, n is too high, then only very small fractions of each spot will be found, and some spots will not be found at all, which can also give misleading results.

A complementary approach to visually examining the images, is to browse the data tables within OME, or download the data to a spreadsheet (see later), in order to count the number of spots found. This enables one to check that this number is biologically sensible, given the type of samples being studied. However, it is worth bearing in mind that there is no 'perfect' thresholding (Young et al., 1998), and an acceptable result is somewhat subjective, depending to a large extent on what is to be done with the data subsequently. The simplest test of adequate thresholding is to see that the results are sensible in terms of where the 'found' spots are and the number of spots found.

Note that in some instances, what appear to be two separate spots may be identified by the algorithm as a single spot; this can occur if at any point in the image stack, there are pixels bridging the gap between the two spots.

Note too that, in the overlay view, the absence of a blue circle around a spot does not mean that it was not picked up, but may simply mean that the *centroid* of that spot is not in the particular Z-section being viewed: a simple way to check this is to move up or down to neighbouring Z-sections, or switch the viewer to display 'all Z' spots together.

If the dataset contains too many images to make it feasible to check them all, then a representative selection should be tested.

Inspection of numerical data

To view the numerical results for a particular image, go to the detailed view of data for that image, e.g. by clicking in the top-left of the image's thumbnail, or by clicking 'Info' when using the viewer to view that image. As described earlier, on the top right are displayed all the module executions performed on that image, and the dates they were performed. In this list are the FindSpots modules and the dates they were run. Click on the run to navigate to a “Module Execution Detail” view. There may be more than one run displayed, if FindSpots has been run more than once on that image; as mentioned above, the correct one can always be verified by its data, and parameters.

A window such as that shown below will appear.

Open Microscopy Environment v2.4.1

Welcome David Schiffmann
No recent project. [create new](#)
Most recent dataset: [UsiRNA201005](#) (Popup)

Home -> -> ModuleExecution Detail

Module Execution: Find spots

Inputs	Outputs
Parameters	Timepoint <input type="checkbox"/>
Pixels	Threshold <input type="checkbox"/>
Stack means	Location <input type="checkbox"/>
Stack geomeans	Extent <input type="checkbox"/>
Stack sigmas	Signals <input type="checkbox"/>
Stack minima	
Stack maxima	
Stack geosigmas	

Merge Outputs

Elapsed Time (R):
Elapsed Time (X):
Elapsed Time (W):
Elapsed Time (T): 13.33294

Dataset:
Image:

Experimenter: David Schiffmann
Status: FINISHED
Error Message:

[View graphic overlay](#)

Outputs

Timepoint [Search within these outputs](#)

[Download as txt](#) | 1 of 4 > >>

ID	TheT	Feature
666964	0	1
666969	0	2
666974	0	3
666979	0	4
666984	0	5
666989	0	6
666994	0	7
666999	0	8
667004	0	9
667009	0	10
667014	0	11
667019	0	12
667024	0	13

Numerical output of FindSpots, accessed via the web interface. To view a graphical representation of the results (the spots found overlaid over the original image), click on ‘View graphic overlay’.

ModuleExecution: Find spots

Address: http://bonedancer.openmicroscopy.org.uk/perl2/serve.pl?Page=D+E...&Web_SBOs_DetailID=5066&Type=D+E...&ModuleExecution

Live Home Page Apple Apple Support Apple Store Mac Mac OS X Microsoft MacTopic Office for Macintosh MDN

ID	Background	CentroidX	CentroidY	CentroidZ	GeometricMean	GeometricSigma	Integral	Mean	Sigma	TheC	Feature
666965	136	72.2	4.7	1850.7			234761	2115	2	1	
666966	136.2	72	4.7	389.4			50714	456.9	1	1	
666967	136.1	72.1	5.1	946.1			112235	1011.1	3	1	
666968	136.2	72.1	5.2	1186.7			132526	1193.9	0	1	
666970	79.4	81.7	8	1408.8			221584	1691.5	2	2	
666971	79.4	81.7	7.8	304.4			47307	361.1	1	2	
666972	79.5	81.9	8.3	900			124096	947.3	3	2	
666973	79.3	82.1	7.9	1184.8			157497	1202.3	0	2	
666975	88.4	41.2	11.3	1570.5			310750	1806.7	2	3	
666976	88.4	41.1	11.2	323.2			66919	389.1	1	3	
666977	88.8	41.1	11.8	892.1			160919	935.6	3	3	
666978	88.6	41.2	11.7	1157.8			201844	1173.5	0	3	
666980	121.2	36.9	9	1740.6			88963	2169.8	2	4	
666981	120.9	36.8	9	285.5			13610	332	1	4	
666982	121	36.8	9.6	893.5			38345	935.2	3	4	
666983	121	36.8	9.6	1273.5			52308	1275.8	0	4	
666985	87.3	56.1	11.6	1330.2			214925	1568.8	2	5	
666986	87.3	56.1	11.4	311.3			48874	356.7	1	5	
666987	87.2	56.3	11.7	868.5			124748	910.6	3	5	
666988	87.4	56.4	12	945.5			131302	958.4	0	5	
666990	66	19.9	11.4	1593.2			201766	2058.8	2	6	
666991	66	19.8	11.3	303			37141	379	1	6	
666992	66.1	19.9	12.1	898			92847	947.4	3	6	
666993	66	19.8	12	902.6			90018	918.6	0	6	
666995	46.5	9.5	15.2	1803.9			326411	2133.4	2	7	

1 of 15 >>>

Inputs

Numerical output of FindSpots, accessed via the web interface (this is the same page as in the previous figure, but after scrolling down).

This page shows the image used as the 'input' to FindSpots, and all the outputs it produced, in a number of tables. For example, the signal intensities (spot integrals) are in the 'Signals' output. Within the web browser, it is usually only possible to view a small portion of each table at a time, and it is often easier to view the data after downloading it and opening it with a spreadsheet program.

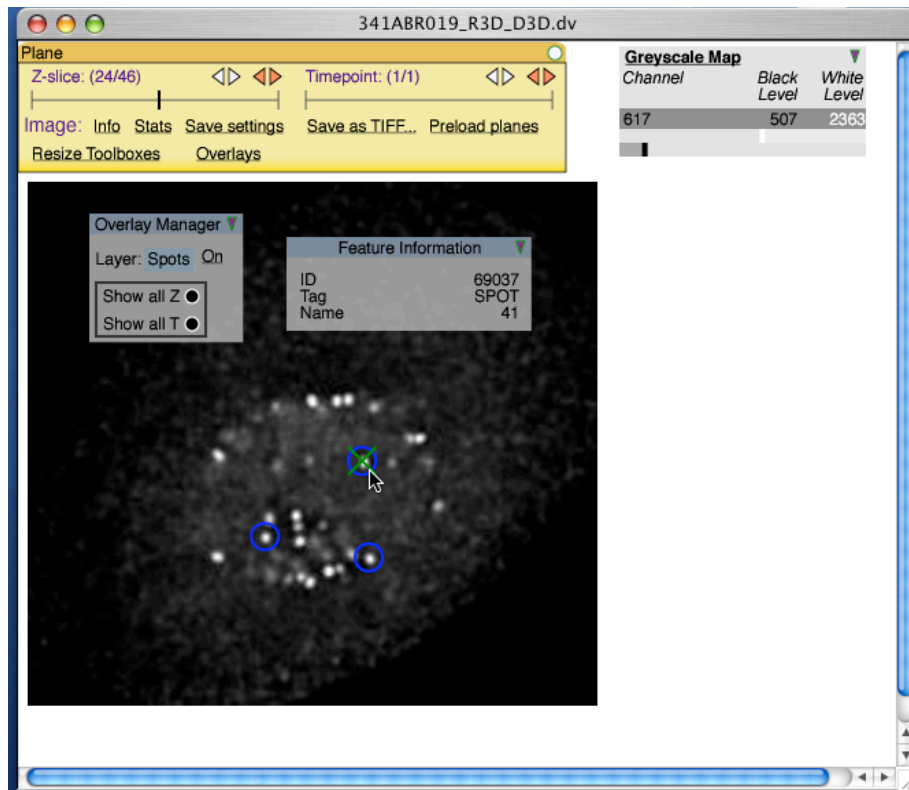
Before downloading the data, it is possible to create larger, 'merged' tables, by selecting some of these outputs at the top of the page and clicking the 'Merge Outputs' button; this will put those outputs into the same table for easier viewing. This table has a row for each spot in each channel in your image. Click the 'Download as text' link to get a tab-separated text file with that data. The first time this is done, it will probably ask if you want to save the file or open it with a certain program. If you tell your browser to open these types of files (text/tab-separated-values MIME type) in Excel, clicking the 'Download as text' will just open them up in Excel from then on.

14. Identifying spots by number

Each spot found by FindSpots (sometimes referred to in FindSpots as a 'feature') has a unique reference number assigned to it, which can be used to look up the detailed statistics (position, total intensity, etc.) about that spot in the output tables. In order to view this reference number, click on one of the blue circles, and a small floating window will appear in the viewer, as shown in the screen-shot below. The important number here is the 'Name'; in the numerical output table, a 'Feature' number is listed for each

spot (see later), which is identical to the 'Name' shown here, and enables one to look up statistics for the spot⁷.

Once this window has been opened, one can simply move the pointer to other spots (i.e. to other blue circles) and the ID number will update automatically. An 'X' mark will always be displayed in the blue circle whose ID number is currently displayed, to ensure that the spot intended by the user, is the one currently displayed.



Each spot has a unique number associated with it.

Note: if 'show all Z' is active (showing spots found in ALL optical sections at once), then it is possible that the blue circle clicked on may in fact not be from a circle whose centroid is in the current optical section. In such a case, the viewer will automatically change to a different optical section, to display the one in which the spot's centroid lies.

15. Importing data into Microsoft Excel – using macros

Introduction

Although it is possible to view and download the numerical data from the various output tables as

⁷ The feature 'ID' number here should be ignored, as it is an internal reference number that provides a unique reference to that feature (spot) within the entire OME database, but it cannot readily be cross-referenced with the spot numbers in the numerical output table, and so will generally not be used by the user.

outlined above, it is possible – and often preferable – to automatically import data from a whole 'run' of FindSpots directly into a Microsoft Excel spreadsheet⁸. This is often preferable and simpler, especially when one needs to obtain and process data from many images; in addition, the tables thus generated contain a more complete set of data than can be obtained by using the 'merge tables' option.

Importing a run of data into Microsoft Excel allows complex further analysis to be performed. In this context, a 'run' means a set of images in any one dataset that has been analysed by FindSpots; any number of images can be analysed together in this way, within the limits of the OME server and Microsoft Excel.

In order to import data into Excel, it is necessary to first open a copy of a special Excel spreadsheet that contains the macros for importing data from FindSpots. This is distributed as part of the Macintosh installer for OME, available via <http://openmicroscopy.org/install/mac-osx.html> ; look for the Excel spreadsheet file called 'OME-Excel.xls' in the 'EXTRAS' folder.

By running the macro as described here, a spreadsheet file will be generated containing the FindSpots data for the specified dataset and the particular 'run' of FindSpots on that dataset, chosen by the user. The spreadsheet file will contain several worksheets, with one worksheet per image file, each containing information and output data for the FindSpots output run on that image file. This includes the image filename, the emission wavelengths, and a table of statistics for each spot in that image, including spot total intensity, mean, and position in 3-D space.

Important notes - before using the spreadsheet

There are a number of important points to bear in mind before using the spreadsheet to import data from OME.

Before running the macros within the spreadsheet, save it under a different name, otherwise it will overwrite the existing contents of the file as it goes along (which one may wish to keep).

Unless one is using the 'original' version of the spreadsheet as downloaded from the OME website, it is also important, before running the macros, to delete all worksheets corresponding to specific images that may be present from the last time it was run. To do this, select the first worksheet, move to the last (use the arrows in the worksheet bar at the bottom-left of the window) and shift-click, then go to 'Edit' menu and choose 'Delete sheet(s)' and click 'OK'. This is important because even if the resulting spreadsheet is saved with a name specific to your data, the file may *to begin with* contain worksheets on it from 'older' analyses, which would be confusing at the time of subsequent analysis if they were not first deleted.

Ensure macros are enabled on your Excel installation; to do this, one may need to (temporarily) lower the Excel security level – see the online Excel Help for information about this.

Excel will likely issue a warning when opening the OME-Excel file, saying: "The workbook you are opening contains macros. Some macros may contain viruses that could be harmful to your computer." Click 'Enable Macros' to continue – otherwise the spreadsheet will not function as needed.

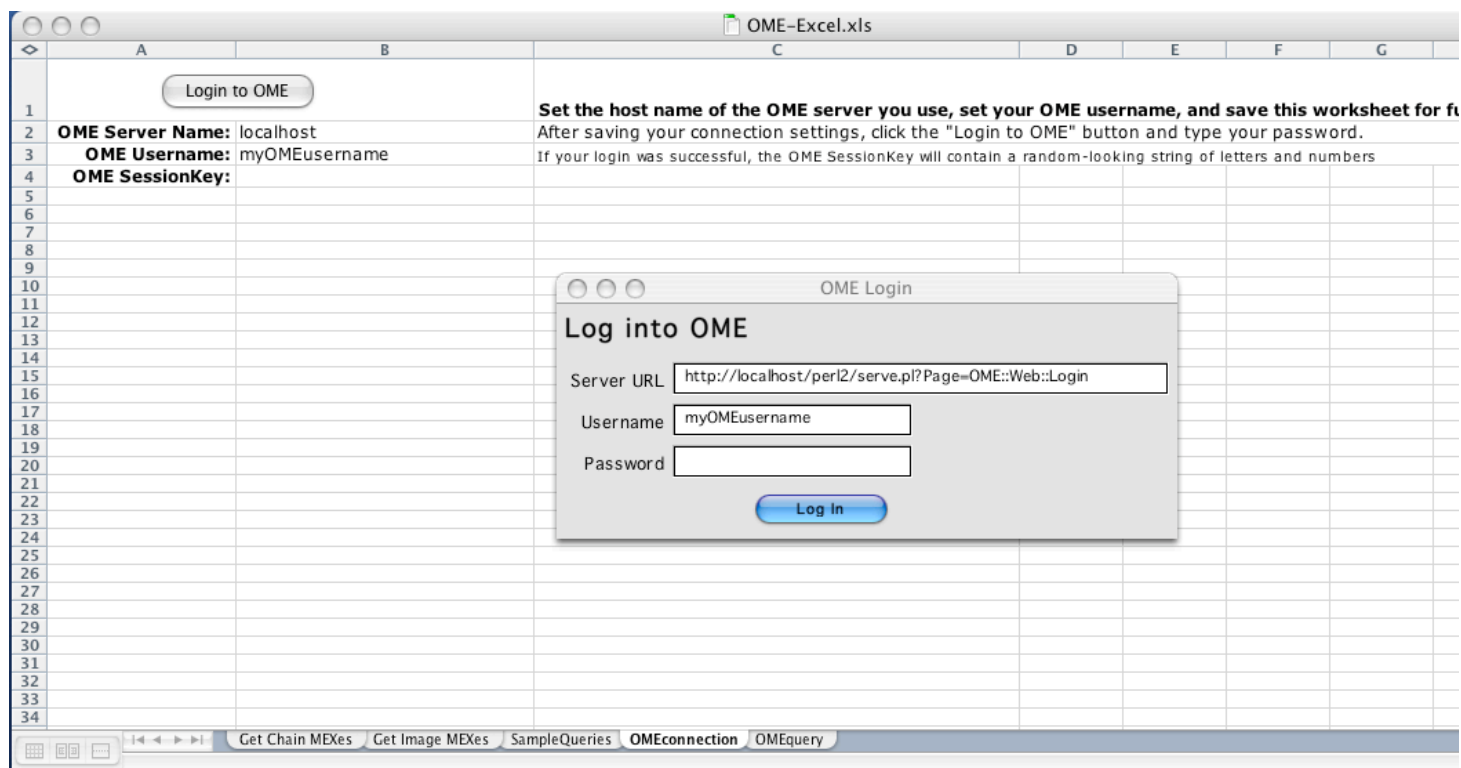
⁸ Note that because of the need to use Microsoft Excel's programming language, this is only possible using Microsoft Excel, and will not work with other spreadsheet programs. Any of the "Download as text" links found in table views in OME pages will download a tab-separated text table to any spreadsheet viewer capable of reading tab-separated text tables.

Using the spreadsheet – connecting to an OME server

After opening the OME-Excel spreadsheet, select the worksheet entitled 'OME connection'. This worksheet will appear when the OME-Excel file is opened. Here one needs to enter the OME server name and userID, and then log in with the password when prompted. This provides the spreadsheet with the current OME 'session key' and allows it to transfer data from the OME server; this must be done prior to other analysis steps.

If one is running the OME server on the same computer as one is using to run this Excel spreadsheet, then 'server name' is simply 'localhost'. The OME Username should be the same one used when installing OME, or any other valid OME user. It is convenient to save the spreadsheet at this point in order to retain the server and username settings for future use. Once these values are entered into the worksheet, click the “Login to OME” button to provide a password, and retrieve a SessionKey from the OME server. The SessionKey will appear in the worksheet if login was successful. The SessionKey looks like a bunch of random letters and numbers.

Note that if you do not import any data for a while, this step will eventually need to be repeated, as one will effectively be 'logged out' due to inactivity, as a security measure.



Importing FindSpots results into Microsoft Excel - logging in to OME. Using the OME-Excel spreadsheet (available from the OME website), select the 'OME connection' worksheet, enter the server address and username, and click 'Login to OME'. A window will appear, in which one can enter one's password, after which the 'session key' will automatically be updated (in spreadsheet cell B4).

Importing data into Excel

The next worksheet to use is 'Get Chain MEXes'. A summary of the steps to follow are provided by the 'Help' button there. First, enter the dataset name in cell B1. When entering the dataset name into the Excel worksheet, it is critical to enter the dataset name avoiding any trailing/leading spaces (these may inadvertently be picked up when one copies/pastes the dataset name from the web browser). Otherwise, Excel will not be able to transfer the data from OME, because the filename Excel is using will not exactly match the one in OME (due to these extra spaces).

Next, click 'Get Chain executions (CHEXes)'. A list will appear to the right of this button, showing all the CHEXes. Each one corresponds to a different 'run' of Find Spots. The more recent the FindSpots run, the greater the ID number (i.e. the lower down the spreadsheet document it is). Click in column H, on the ID number corresponding to the FindSpots run you would like to extract data from. Then click the 'Get MEXes' button.

Then, as it says, click on 'Get All results' to get all the results, or select a subset of them (column E 'IDs') and click 'Get results for selection'. This may sound complicated, but should be clearer after trying it a few times!

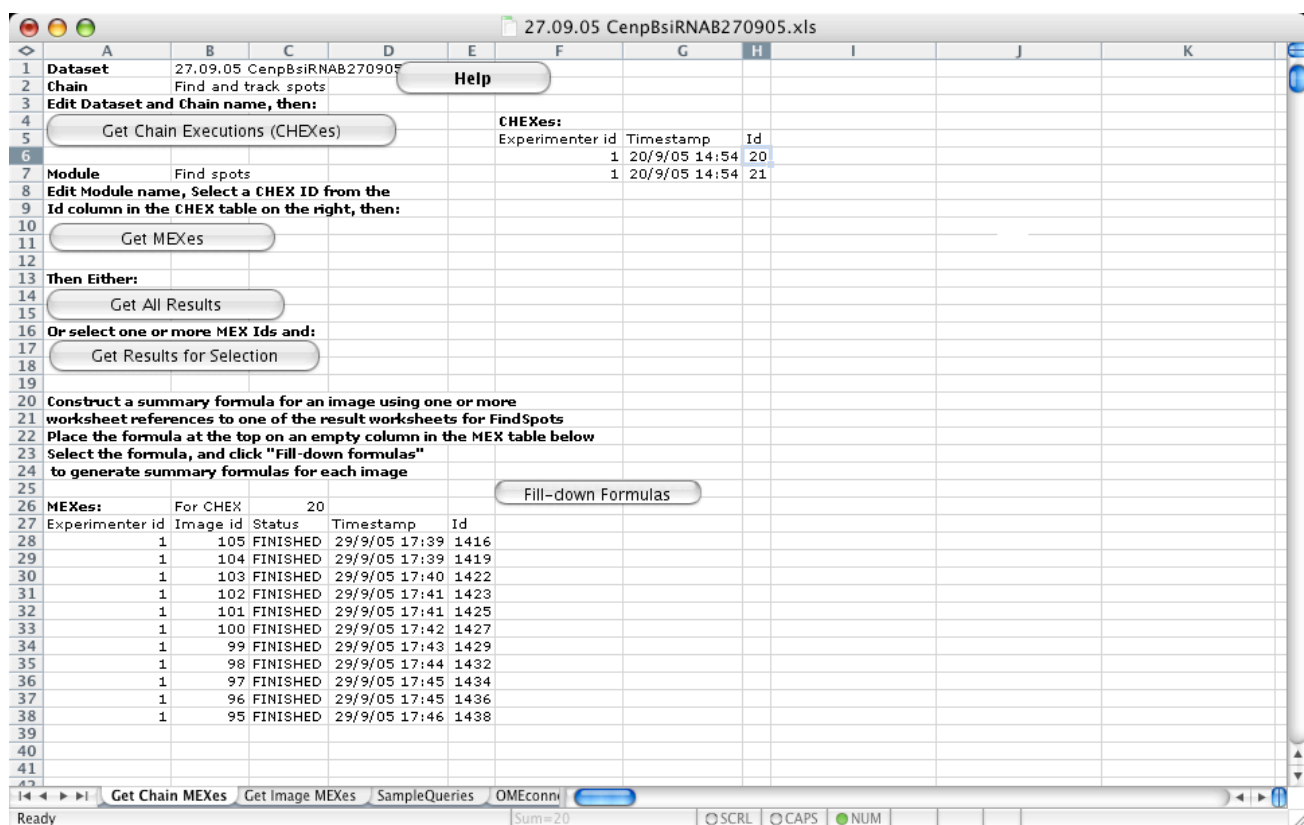
Then wait whilst it queries the OME server and generates the spreadsheet. This can take from a few minutes to several hours (or days, potentially, for extremely large datasets!), depending on the number and nature of the images in the dataset and, but one's personal computer can still be used whilst this is happening.

27.09.05 CenpBsiRNAB270905.xls

	A	B	C	D	E	F	G	H	I	J	K
1	Dataset	27.09.05 CenpBsiRNAB270905									
2	Chain	Find and track spots			Help						
3	Edit Dataset and Chain name, then:										
4	Get Chain Executions (CHEXes)					CHEXes:					
5						Experimenter id	Timestamp	Id			
6						1	20/9/05 14:54	20			
7	Module	Find spots				1	20/9/05 14:54	21			
8	Edit Module name, Select a CHEX ID from the										
9	Id column in the CHEX table on the right, then:										
10	Get MEXes										
11											
12											
13	Then Either:										
14	Get All Results										
15											
16	Or select one or more MEX IDs and:										
17	Get Results for Selection										
18											
19											
20	Construct a summary formula for an image using one or more										
21	worksheet references to one of the result worksheets for FindSpots										
22	Place the formula at the top on an empty column in the MEX table below										
23	Select the formula, and click "Fill-down formulas"										
24	to generate summary formulas for each image										
25						Fill-down Formulas					
26	MEXes:	For CHEX									
27											
28											
29											
30											
31											
32											
33											
34											
35											
36											
37											
38											
39											
40											
41											

Get Chain MEXes Get Image MEXes SampleQueries OMEconnection OMEquery

Importing FindSpots results into Microsoft Excel – How to specify the desired dataset and FindSpots run, to import data from (1). Go to the ‘Get Chain Mexes’ worksheet, enter the dataset name, and click ‘Get Chain Executions (CHEXes)’. The worksheet will look similar to that shown here. In columns F-H will appear a list of ID numbers, each corresponding to one FindSpots run. Select one of the cells in column H corresponding to the desired run, and click ‘Get MEXes’



Importing FindSpots results into Microsoft Excel – How to specify the desired dataset and FindSpots run, to import data from (2). One will now see a window like this. The simplest next step is to click on 'Get All results' to begin the import of numerical FindSpots results (spot size, total intensity, etc.) for all images in the dataset. One can then enter formulae in columns F and/or those to the right, and click 'Fill-down formulas' to automatically generate the same formula for each image in the spreadsheet (e.g. for each image in that FindSpots run), but modified to refer to each particular image (worksheet) in turn.

Layout of imported data

When import of data has finished, look through some of the worksheets to familiarise yourself with the layout of the data.

Feature	Module	execution	TheT	Threshold	TheX	TheY	TheZ	Volume	SurfaceArea	CentroidX	CentroidY	CentroidZ	GeometricMean	Integral	Mean	CentroidX	CentroidY	CentroidZ	Geom
11	27605	1437	0	992	10.4	54.4	7.2	39	37	10.4	54.4	7.3	190.7	7450	191	10.4	54.4	7.1	
12	27607	1437	0	992	12	77.5	3.8	31	31	12	77.5	3.8	161	4996	161.2	12	77.5	3.8	
13	27608	1437	0	992	88.7	34.8	5.1	36	31	88.6	34.8	5.2	415.6	15094	419.3	88.6	35	5.1	
14	27610	1437	0	992	85.4	48.7	6.2	44	37	85.4	48.7	6.2	442.5	19528	443.8	85.3	48.6	6.2	
15	27612	1437	0	992	90.8	40.2	10.8	32	31	90.6	40.2	11	543.7	17504	547	90.4	40	11.4	
16	27614	1437	0	992	58.3	43.3	9	35	30	58.3	43.3	9	532.5	18668	533.4	58.2	43	8.9	
17	27617	1437	0	992	70.3	51.1	11.7	49	40	70.3	51.1	11.6	501.7	24724	504.6	70.2	50.9	11.4	
18	27618	1437	0	992	26.1	100.9	11.7	42	41	26.1	100.9	11.7	137.6	5786	137.8	26.1	100.9	11.6	
19	27619	1437	0	992	74.6	37.4	12.5	34	28	74.6	37.5	12.5	452.7	15423	453.6	74.5	37.6	12.6	
20	27620	1437	0	992	48.2	43.9	12.5	32	28	48.2	43.9	12.5	536.3	17173	536.7	48.4	43.7	12.4	
21	27621	1437	0	992	23.8	57.5	16.5	51	50	23.8	57.4	16.6	253.9	12976	254.4	23.8	57.4	16.6	
22	27622	1437	0	992	52.1	32.5	13.9	33	30	52.1	32.5	13.9	508.9	16810	509.4	52.1	32.7	14.3	
23	27623	1437	0	992	68	44.5	14.5	34	32	68	44.5	14.5	545.2	18558	545.8	68	44.4	14.4	
24	27624	1437	0	992	111.5	61	14.2	42	37	111.6	61	14.3	541.2	22763	542	111.6	60.8	14.1	
25	27625	1437	0	992	74.9	66.9	13.5	30	27	75	66.9	13.6	347.4	10472	349.1	74.9	66.9	13.5	
26	27626	1437	0	992	117.3	51	15.2	41	35	117.3	51	15.2	442	18165	443	117.2	51.2	15.1	
27	27627	1437	0	992	119.7	64.2	15.9	44	41	119.6	64.2	16.1	500.9	22149	503.4	119.8	64.3	15.7	
28	27628	1437	0	992	113.3	73	16.9	39	33	113.3	73	16.9	385.4	15081	386.7	113.3	72.8	16.8	
29	27629	1437	0	992	47.8	36.9	17.7	34	30	47.8	36.9	17.7	535.8	18235	536.3	48	36.8	17.6	
30	27630	1437	0	992	38.3	37	18.3	58	49	38.3	37	18.3	457.8	26646	459.4	38.2	36.7	18.1	
31	27631	1437	0	992	111.1	44	18.1	34	31	111.1	44	18.2	345.8	11772	346.2	111.2	44.2	18.1	
32	27632	1437	0	992	69	51.2	24.9	378	273	69.1	51.2	24.9	589.6	223518	591.3	69.8	50.5	23.7	
33	27633	1437	0	992	110.8	67.6	19.2	33	33	110.9	67.6	19.2	466.1	15458	468.4	110.7	67.5	18.9	
34	27634	1437	0	992	8.2	68.4	22.3	47	47	8.2	68.4	22.1	156.6	7385	157.1	8.2	68.4	22.3	
35	27635	1437	0	992	20	77.7	19	32	29	20.1	77.6	18.9	169.5	5439	170	20.1	77.6	19	
36	27636	1437	0	992	44.4	42.1	24.1	51	43	44.5	42.2	24	500.6	25601	502	44.3	41.9	24.2	
37	27637	1437	0	992	128.9	54.8	23.4	33	31	128.9	54.8	23.5	406.5	13511	409.4	128.8	54.9	23.3	
38	27638	1437	0	992	70	59.7	24.1	53	44	70	59.7	24.2	577.6	30696	579.2	69.8	59.7	24	
39	27639	1437	0	992	38.3	48.2	25	56	45	38.3	48.2	25	457.4	25641	457.9	38.3	47.8	24.8	
40	27640	1437	0	992	92.6	64.1	24.8	43	37	92.6	64.1	24.7	548.9	23626	549.4	92.8	64.1	24.6	
41	27641	1437	0	992	41	33.4	25.8	34	32	41	33.4	25.8	441.7	15041	442.4	41.3	33.3	25.6	
42	27642	1437	0	992	73.5	38.5	25.4	31	27	73.5	38.5	25.4	561.1	17433	562.4	73.5	38.7	25.3	
43	27644	1437	0	992	95.9	54.7	25.6	37	32	95.9	54.7	25.6	590.7	21873	591.2	95.8	54.8	25.3	
44	27646	1437	0	992	112.6	55	27.4	62	55	112.6	55	27.6	485.2	30192	487	112.7	55.1	27.4	
45	27647	1437	0	992	17	57	24.9	32	29	17	57	25	265.3	8518	266.2	17	57	24.9	
46	27650	1437	0	992	113.5	76.2	25.5	41	36	113.5	76.2	25.4	405.7	16720	407.8	113.6	76.1	25.3	
47	27651	1437	0	992	55.2	33.9	29	77	69	55.3	34	29.2	486.1	37689	489.5	55.1	34.1	28.8	
48	27653	1437	0	992	66.9	69.8	28.8	38	37	67	69.7	28.8	361.1	13752	361.9	67	69.7	28.9	
49	27655	1437	0	992	54.1	42.1	27.4	32	28	54.1	42.1	27.5	555.6	17820	556.9	54.2	42.2	27.8	
50	27657	1437	0	992	21.8	83.7	30.5	79	70	21.8	83.6	30.6	151.6	12006	152	21.8	83.7	30.5	
51	27659	1437	0	992	46	24.8	28	34	31	46	24.8	28	316.8	10787	317.3	45.9	25	28	
52	27661	1437	0	992	74.4	87.6	28.1	38	35	74.4	87.6	28.1	216.4	8240	216.8	74.4	87.6	28.1	
53	27663	1437	0	992	41.5	37.8	29.3	55	46	41.5	37.8	29.3	416.6	22944	417.2	41.5	38.1	29.3	
54	27665	1437	0	992	52.6	46.4	30.6	42	38	52.6	46.4	30.6	541.3	22786	542.5	52.7	46.3	30.3	
55	27667	1437	0	992	57.9	62.3	31.6	70	60	57.9	62.3	31.6	490.5	34471	492.4	58.2	62.1	31.5	
56	27668	1437	0	992	117.9	68	30.8	50	42	117.9	67.9	30.8	556.9	27874	557.5	118.1	67.8	30.6	
57	27670	1437	0	992	120.3	58.1	32.5	41	40	120.3	58.2	32.4	484.2	19898	485.3	120.2	58.4	32.3	
58	27672	1437	0	992	74.2	57.2	34.5	93	77	74.2	57.1	34.4	561.3	52347	562.9	74.4	56.8	34.3	
59	27674	1437	0	992	93.5	71	32.9	30	27	93.5	70.9	32.9	452.1	13656	455.2	93.6	70.8	32.9	
60	27676	1437	0	992	89.4	62	34.3	37	32	89.4	62	34.3	589.7	21852	590.6	89.6	61.9	34.4	
61	27678	1437	0	992	127.4	67	35.6	52	45	127.4	67	35.6	505.8	26345	506.6	127.5	67.2	35.4	
62	27680	1437	0	992	114	65.5	39.3	76	69	114	65.5	39.2	503	38307	504	114	65.7	39.6	

Example of layout of imported data. A separate worksheet is generated for each image, listing the FindSpots results, as shown here. At the top-left of the image (red box) is a summary of the filename and the emission wavelengths. Each row (thick blue arrow) corresponds to one spot. The data is divided into blocks of columns, as shown by the red arrows; the thick red arrow corresponds to basic spot data as defined by the thresholding, whereas the thin red arrows show data specific to each wavelength in turn, in the order shown in the box. Hence A corresponds to 457 nm fluorescence emission, B to 528 nm, etc.

For each image, a separate worksheet is generated. In each such worksheet, there is a header with information common to all the spots in that image, and actual spot data laid out in columns, one column per 'channel index number'/emission wavelength, in the same order as given in each worksheet, as shown in the screen-shot above.

For example, in the above worksheet, the left-most block of data corresponds to channel 0 (457 nm emission), the next block to the right corresponds to channel 1 (528 nm) and the right-most block to channel 2 (617 nm). Only the first block is shown, for reasons of space.

Processing the imported data

To further process the Excel spreadsheet thus generated, go to the 'Get Chain MEXes' worksheet; this is where formulae can be generated and automatically applied to all worksheets, and is a convenient place to generate a summary table from all the images in the dataset, for statistical analysis or plotting.

Before preparing formulas, it may be helpful to make a separate list of the dataset this is from, and what the different emission wavelengths correspond to (e.g. what antigen is labelled in each channel), and the 'channel index number' (0, 1, 2 etc). Then, use this to prepare formulas as required. Any formulas that need to refer to the worksheet, should be generated using an arbitrary worksheet/filename and placed in one cell as an example. Clicking on the 'Fill-down formulas' button will then generate versions of the formula corresponding to each of the worksheet name, forming a column with a suitably modified formula in each cell. Repeat this to generate a new set of formulas in another column, as needed. This should be done before any spaces are inserted between rows, otherwise the automatic formula-generation will stop when it reaches one of these spaces.

The way in which the data is analysed next, depends on the needs of the user. For example, for any one image, it is possible to generate scatterplots of volume vs. mean intensity, or x-position vs. total spot intensity; or to sum the total intensity of all spots in the image, in each of the different wavelengths.

Post-import checks

After importing data into Excel and preparing the formulas and a summary table, it is a good idea to go through this table and look for any potential problems. It may be helpful to generate a column giving the total number of spots per image, to help detect images with an unusually large or low number of spots, which may indicate a problem with the image. Other problems could include the presence of images that should not be there (as judged by their filename).

Move any rows (each corresponding to one image) to be ignored, to another part of the spreadsheet or delete them. It may be the case that in some images, certain spots could not be excluded by cropping (due to the restriction to a rectangular cropping shape, or otherwise) and these will need to be manually removed – within FindSpots WebUI, find the unwanted spots' ID, and then delete these from the data table in Excel; however it is far easier to simply avoid using such images, or crop them suitably before analysis.

16. References

I.T. Young, J.J. Gerbrands, and L.J. van Vliet, [Fundamentals of image processing](#), in: V.K. Madisetti, D.B. Williams (eds.), *The Digital Signal Processing Handbook*, CRC Press, Boca Raton FL, 1998, 51.01-51.81.