

# A Quick Help to the Standard Procedure

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## Contents

<b>1 Introduction</b>	<b>1</b>
<b>2 Import</b>	<b>1</b>
<b>3 Correction</b>	<b>2</b>
<b>4 Object Search</b>	<b>3</b>
<b>5 Tracking</b>	<b>4</b>
<b>6 Masking and Statistical Filters</b>	<b>4</b>
<b>7 Statistic</b>	<b>5</b>

## 1 Introduction

The following steps should give you a quick help to get objects (and tracks if necessary) out of data. If you experience any problems with any step or if the function you need is not covered here, read the respective chapter in the manual to get a better understanding. It is advised to set up Grid Computing before but since this is rather complicated we assume that you either use a machine where it is already set up (like a workstation in a lab already using MotionTracking) or wait until the calculation is done locally on your own machine.

If you can't find any of the mentioned buttons check the first Chapter on the user interface.

## 2 Import

In this quick guide we have to assume that you already have a project file and the import was done already because the procedure depends on your image source and can't be summarized in two sentences. If this is not the case, read the chapter "Import of Data and Project Management" in the manual.

### 3 Correction

Additionally we assume the presence of two projects used to correct the image:

- empty images with the microscope’s laser turned on and off
- images with “beads” which reflect the whole spectrum and can be used to correct the shift between images taken with different laser frequencies

These are also covered in the “Import” chapter in the manual.

If you have several projects with different corresponding beads and laser screens, make sure all of them are imported correctly, because it makes no sense to use a wrong correction file.

These project files contain the images we need to correct the systematic errors produced by the microscopes.

1. Open the project showing nothing but the laser turned on and off. Go to “Processing → Uneven Illumination/Chromatic Shift → Calculate Noneven Illumination”. An Save Dialog will appear, save the .ilm file.
2. Open the project with the beads. Apply the illumination correction calculated in the previous step by clicking on the “Illum. Correction” button in the lower left part of the user interface and selecting the “.ilm” file in the appearing dialog.
3. Use the parameters shown in Figure 1 to calculate objects by selecting “Objects → Object Search Parameters” to set up the parameters and then “Objects → Find Objects” (Grid Computing advised). Save the “.chs” file when asked.
4. When those steps are done, open any of the projects with real image data and click on the “Illum. Correction” and “Chs. Shift Corr.” buttons in the lower left part of the interface. Select the “.ilm” and “.chs” you created respectively in the steps 1 and 2.

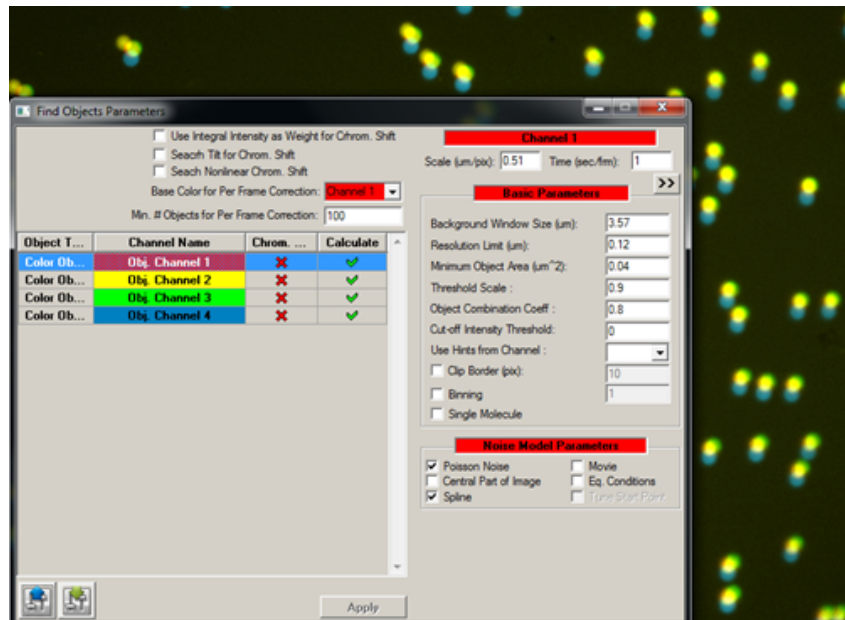


Figure 1: Typical object search parameters to find beads. Note that the Threshold Scale and the Object Combination Coefficient may vary significantly.

You can view the corrected image by selecting the “Show Corrected” Checkbox in the left part of the interface, which you should do to find out if everything went right. If not, either the beads and laser screens are not relevant for the set of frames or the object calculation in the beads project went wrong. If the latter is the case read the chapter on “Object Search” and recalculate, but generally the parameters in the example work fine.

## 4 Object Search

Now you have to calculate the vesicles. For the channels showing cargo vesicles like those typically marked by EGF or LDL and others of comparable size you can use the parameters shown in Figure 2, for Nuclei those in Figure 3. You must to adjust the “Threshold Scale” parameter to you individual project. To do this simply play around with it until the result is reasonable. If several objects are merged together or you see several objects where only one is present in the picture you must alter the “Object Combination Coefficient”. You will have to experiment around with the values a bit at this point, there is no way around it.

It is highly advised to use the “Objects → Find Objects in Spline Contour” to calculate the selected frame individually to check if the object parameters fit before calculating the whole image frame set with “Object → Find Objects”. Check Figure 4 for reference.

Note: Remember to set up the parameters for each channel.

In the following Figure 2 and Figure 3 the right side (where the fields labeled with “Parameters” are) is the important one.

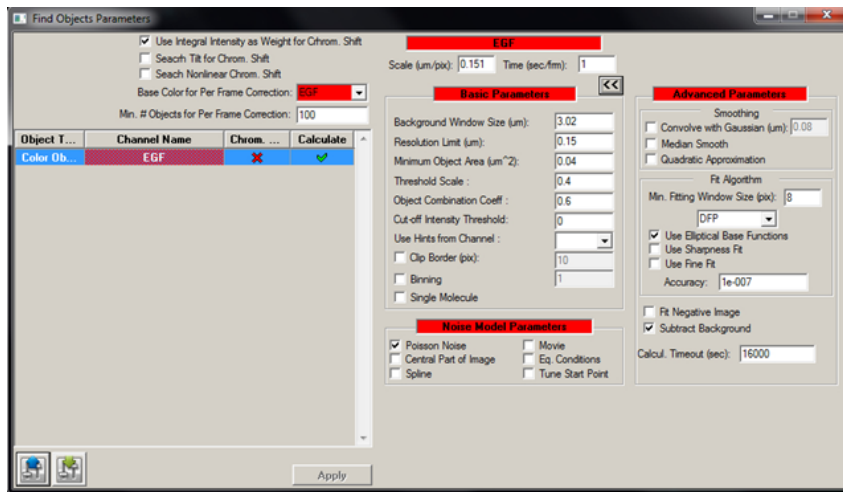


Figure 2: Parameters for small vesicles.

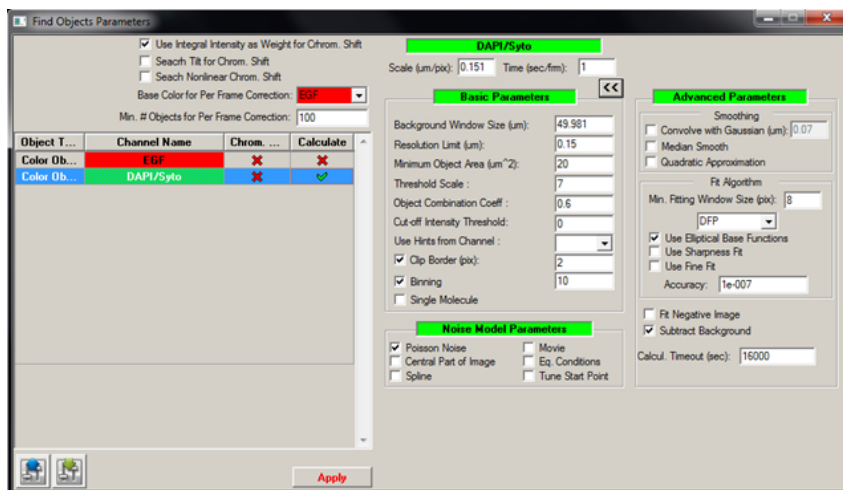


Figure 3: Parameters for large vesicles.

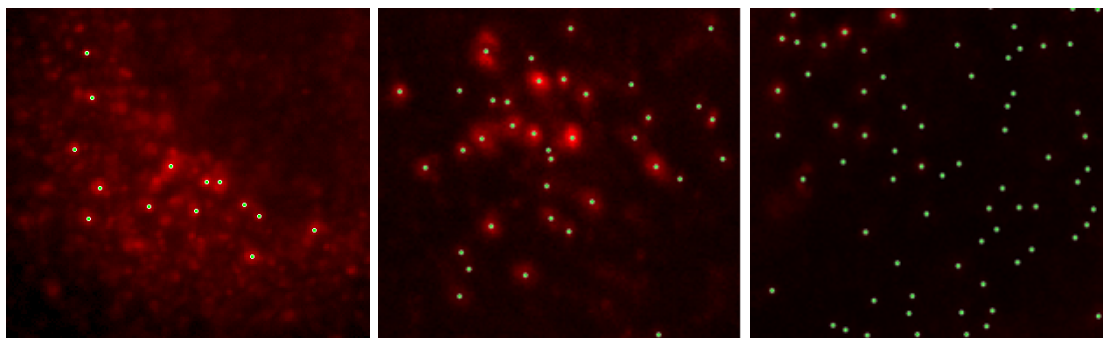


Figure 4: Different results from left to right: too few objects, reasonable result, too many objects

## 5 Tracking

If you have a living cell movie or something comparable where you are interested in the information about the movement of vesicles proceed as described below. Ignore this section if you have pictures of a fixed cell or similar.

1. Set up track search parameters: Open “Tracks → Track Search Parameters”. Use the default parameters (shown in Figure 5) and click “Automatic Params” to generate automatic values for Min. Intensity, Min. Area, Critical Intensity and Total Score Threshold.

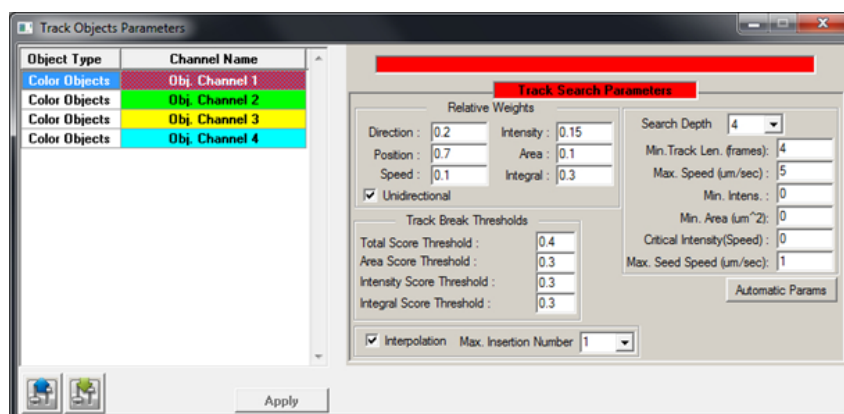


Figure 5: Track Search Parameters

2. Calculate Tracks: Select “Tracks → Find Tracks” and wait until done. It’s advised to do this locally for single projects because the calculation time is usually less than the data transfer time.
3. Once done, save everything.

Now the data mining process is done. Everything else is based on this data.

## 6 Masking and Statistical Filters

Certainly there are some restrictions to the data found so far. Some objects are bound by some characteristics, for example there is a minimal size of a nucleus. You can enter these restrictions when you open “Statistic → Object Statistic Filter Parameters”.

Example: If Channel 2 is your nuclei channel, you can put a minimal “object radius (um)” of 3.

Additionally you can access the mask from the same window where you enter the filter parameters. You can use the mask to cut off parts of the picture based on the intensity on any channel. To learn how to set up the mask read the “Masking” chapter of the manual.

## 7 Statistic

Since this part is very complex and very important you should read the chapter on “Statistics” in the manual. Still, one simple example will be covered here to give you the feel how to use the Batch Statistic Window.

While a project with calculated Objects (and Tracks) is open:

1. Open “Statistic → Batch Statistic → Open Batch Statistic Window”. You will see a table with two columns. Ignore the second one for now. In the first column you will see file names of your image data. For each image in the project there will be a individual row.
2. Go to “Image Set Analysis → Object Characteristic”. Select Size and click ok in the appearing dialog. An additional column will appear which is labelled “Mean: Size (Channel 1)” or similar.
3. Now click execute. For each image (= row) you will get a number in the column, which is the result of the respective statistic - mean object size in this case.
4. Simply add the columns you need in a similar fashion to get the statistics you are looking for. A description on some of the object characteristics can be found in the “Statistics” Chapter in the manual.
5. You can fill out the second column, the Sequence Value, at any point. This can happen either manually or automatically. The information provided in this column is essential for many statistic or data processing operations like “Oligo Correlation” and it is used to label any results. To Edit a single cell simply double click on it, to edit multiple select them and “Right Click → Set Name”. MotionTracking uses the format “Gene Symbol; OligoID/RNAi” providing two slots to save information separated by a semicolon. Technically you can enter any data in these two slots if it suits you better, but remember that the user interface will still assume that the first entry is means Gene and the second Oligo when it comes to the mentioned statistics.