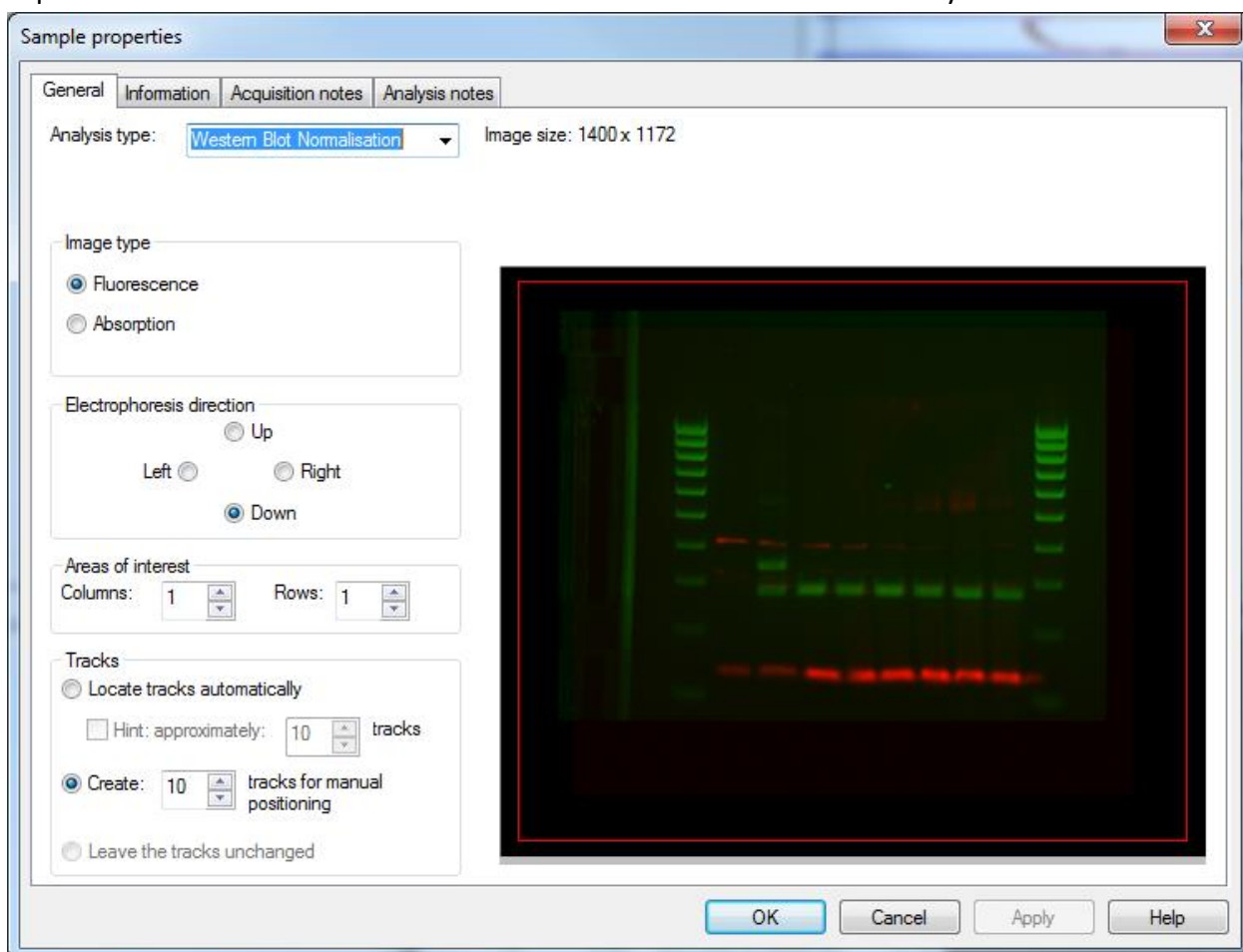


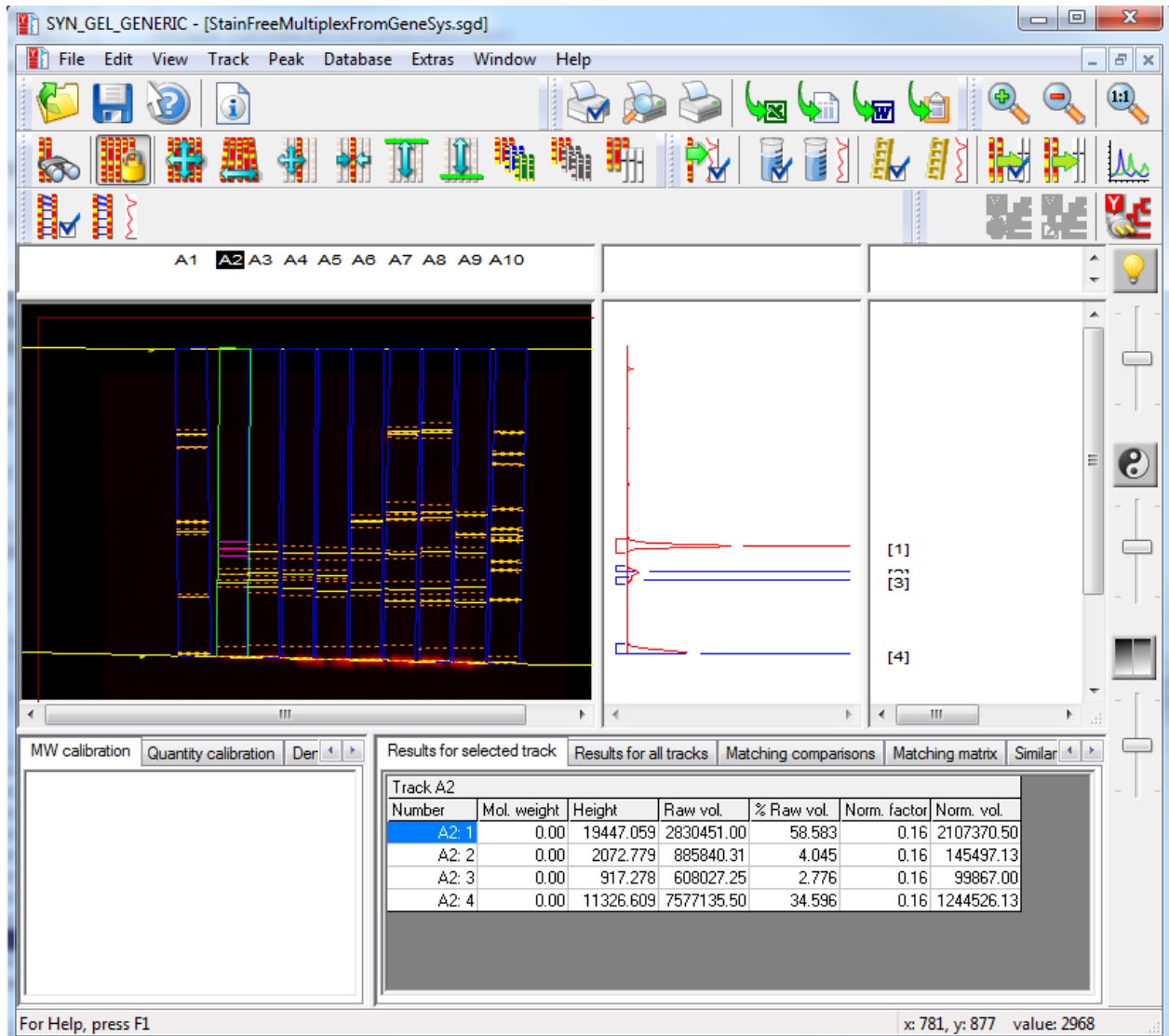
Quick Guide to Western Blot Normalisation in GeneTools

- 1) Capture your total-protein channel (for instance stain free blot) and chemi (or other) blot (protein of interest) in GeneSys and form a multiplex file as described in the GeneSys Quick Guide.
- 2) Run GeneTools and open your multiplex file. If the file is a multiplex capture and contains a "Criterion TGX Stain Free (Membrane)" capture the GeneTools Sample properties dialog will automatically display the analysis type "Western Blot Normalisation". If the multiplex file does not contain a Syngene Criterion TGX capture the user will need to select Western Blot Normalisation manually.



- 3) When the user presses OK the lanes and bands are detected, a normalisation channel is assigned and a normalisation standard track is assigned. For stain free experiments, the normalisation channel is assigned as the gel that contains the dye "Criterion TGX Stain Free (Membrane)", for other captures the user will need to assign the normalisation channel.

- 4) The normalisation standard track is assigned as track 2 by default.



- 5) The normalised volumes are displayed in the results table. These are based on the raw volume and the normalising factor. The normalising factor being the ratio of the non-standard track to the standard track.
- 6) If required a normalised quantity can be calculated based on an assigned quantity for a known/ designated peak. For example if a peak is known to have a quantity of 100ng this can be assigned and the normalised quantity will appear based on the quantity calibration and the normalising factor.

Track editing

In other forms of multiplex analysis it is assumed that all the captures have happened while the sample remains in the darkroom. With western blot normalisation analysis this is not the case and so we cannot be certain that two captures will exactly match. This means we have to allow independent movement of the track boundaries between the two images. So for instance when the tracks are repositioned in one

channel of a multiplex analysis, this movement is copied to all other channels. However with normalisation the question is asked “Do you want to apply these changes to other channels?” It is still necessary to force some editing to be copied to all channels in normalisation analysis. So the adding or deletion of tracks will always be copied to the other channels.

Setting Normalisation Standards

When a user first opens a Normalisation multiplex file the Normalisation settings are:

- 1 **Normalisation Channel** -> The channel with the Normalisation Blot (as defined by GeneSys) otherwise the first gel.
- 2 **Normalisation Track** -> Track 2.
- 3 **Normalisation Method** -> Track Profile.

The **normalisation channel** can be altered by selecting a channel from a drop-down list presented when the Normalising Channel Selection Button is pressed.



Normalising Channel Selection Button.

The **normalisation track** can be changed by selecting a track in the normalisation channel and then pressing the set normalising standard button.

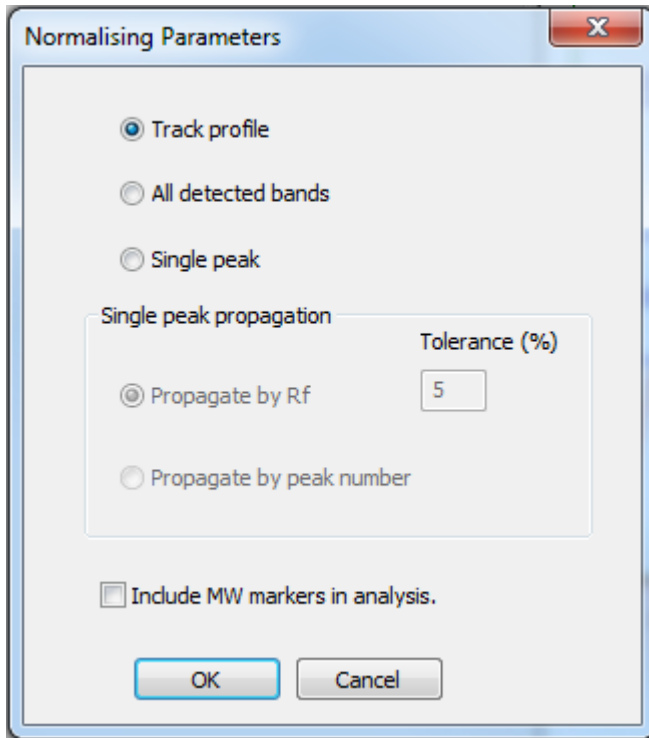


Set Normalising Standard Button.

The **normalisation method** can be altered by selecting one of the options in the normalisation parameters dialog. The normalisation dialog appears when the Normalising Parameters button is pressed.



Normalising Parameters Button.



Track profile:- uses every point in the track to calculate the ratio.

All detected bands:- uses the sum of the volumes of the detected bands to calculate the ratio.

Single peak:- uses a single band in each lane to calculate the ratio.

Propagate by Rf:- If the normalisation is performed by the single peak method, this becomes enabled. The single peaks in lanes other than the standard will be set by their migration value (Rf).

Tolerance (%):- If the Propagate by Rf is enabled and selected , this box sets the tolerance for matching peaks to propagate through the gel.

Propagation by peak number:- If the normalisation method is the single peak method, this becomes enabled. The single peaks in lanes other than the standard will be set by their peak number.

Include MW markers in analysis:- If the user wishes to include any MW markers in the analysis they can tick this box. It is set off by default.

There are a number of means to change this original set-up:

- 1) Select a new normalisation channel:- The normalisation method remains the same, the standard track is set to the first one and, if the single peak method is currently selected, the standard peak is the first peak of the first track.

- 2) Select a new normalisation track:- The normalisation method remains the same, the standard track is the one you have selected, if the single peak method is currently selected, the standard peak is the first peak of the first track.
- 3) Select a new normalisation peak (only applicable when normalisation method is single peak):- The normalisation method remains the same, the standard track is the one you have selected, the standard peak is the peak you have selected.
- 4) Select a new normalization method:- The normalisation method is that selected, the standard track is the one you have selected, if the single peak method is currently selected, the standard peak is the first peak of the standard track.

Enabling/ disabling of functions

The normalisation functionality is not always available in GeneTools. The following will disable the normalisation functions/ buttons.

- 1) The sample type is something other than “Western Blot Normalisation”
- 2) Going into Edit mode by selecting “Move All Tracks” or another of the editing functions.

The “Set Normalisation Standard” button (and pop-up menu item) will be disabled if:

- 1) The selected gel is not the normalisation reference gel.

The “Assign Normalisation Standard” pop-up menu option will be disabled

- 1) The selected gel is not the normalisation reference gel.
- 2) The selected Track is not the normalisation reference track
- 3) The normalisation method is not “Single Peak”