# Multi-time setup

1. Make a database where the final time-lap series will be stored. Best place is a mobile hard disk connected to a fire wire or a USB 2 port (a USB 1 connection will be to slow)
2. Make a folder where you can store the temporary files. Best place is a mobile hard disk connected to a fire wire or a USB 2 port (a USB 1 connection will be to slow)
3. Choose the right lasers, lens, filters, scan-settings (zoom, scan-speed, uni- or bi-directional etc.) and settings for a possible Z-scan.
4. Switch on the 633-laser if you are planning to do an autofocus on the glass-slide.
5. Save the configuration you want to use as a multi-track configuration with the name 'current' (overwrite→yes)
6. Go to 'Stage and focus Control' and mark the locations of the cells you want to follow with the 'Mark Pos.'-button.

The following steps can be used if you want to autofocus on the glass-slide.

7. In 'Stage and Focus Control' go to the first cell and write down the Z-value (Z1).
8. In control panel check the 633–laser, set it to 5% and use the next settings: HFT488/543/633, mirror, mirror, LP505, ch 2
9. Scan continuously and bring down the objective slowly until you get the reflection of the glass.
10. Make a Z-range and find the middle of the region with the reflection (Z2).
11. The difference of Z1 and Z2 will be used later on as the Z-offset in the multi-time macro.

Now start the Multi-time macro and check the following settings:
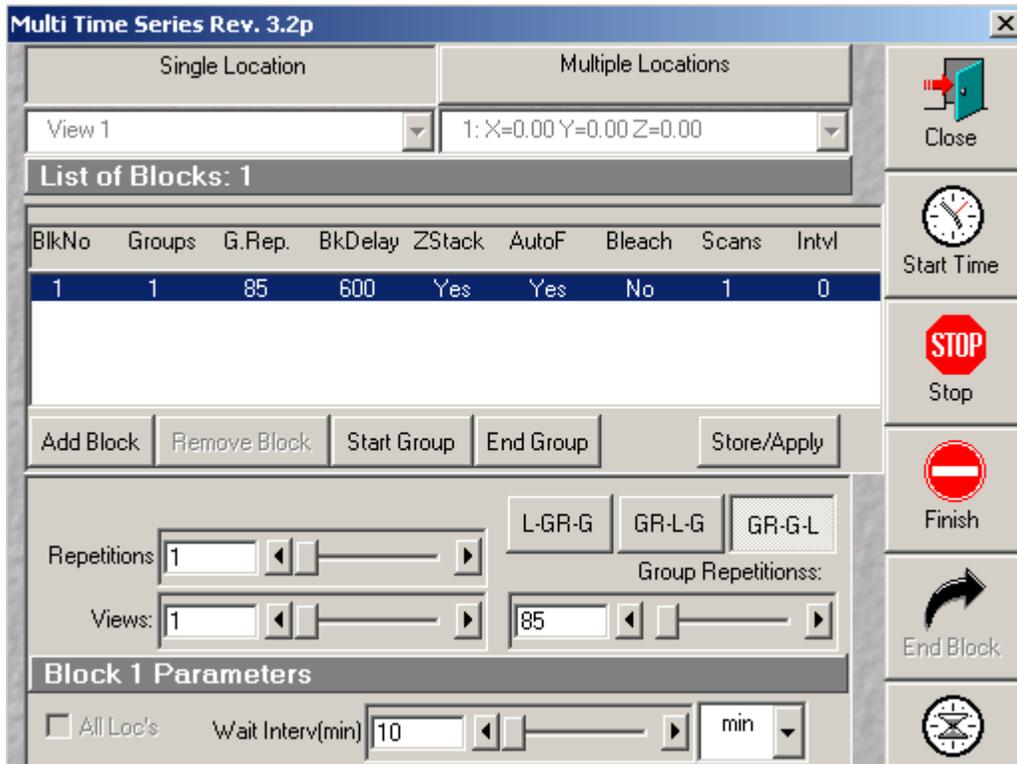
12. Select Single Location.
13. Fill in the Wait interval (10 min in this example)
14. Fill in the number of Group Repetitions (85 in this example)

Check the autofocus settings
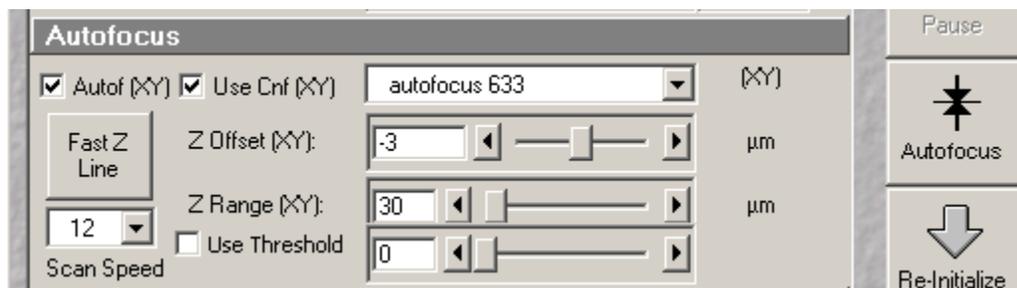
15. Check Autof (XY) and Use CNF (XY). Autofocus 633 must be on screen. Fill in the previous acquired Z-Offset at stage 11 (normally with cells attach to the glass a value between –2 and –4), Z-range 30 and Fast Z Line with speed 12

The Configuration settings

Check and/or select the following settings:

16. Select Multi Track configuration 'current'
17. Normally Number of Scans must be 1
18. It is possible to make more scans (raise the 'Number of Scans') with a certain time interval., for example:
    Location 3 has a number of Scans =2 with a Time Interval of 10 seconds, every other location has a number of Scans=1. The first 2 locations wil be recorded with only 1 scan, the third will be recorded once, then a pause for 10 seconds and then recorded a second time before going to location 4.
19. Check Z-Stack if set earlier (stage 3)
20. Single File Output must be Unchecked
21. Fill in a Base File Name
22. Select the data base were you want to store the final time lapse series with the 'Image Data Base' button (stage 1)
23. Select the folder were you want to store the temp files with the 'Tmp Image Folder'

Final settings
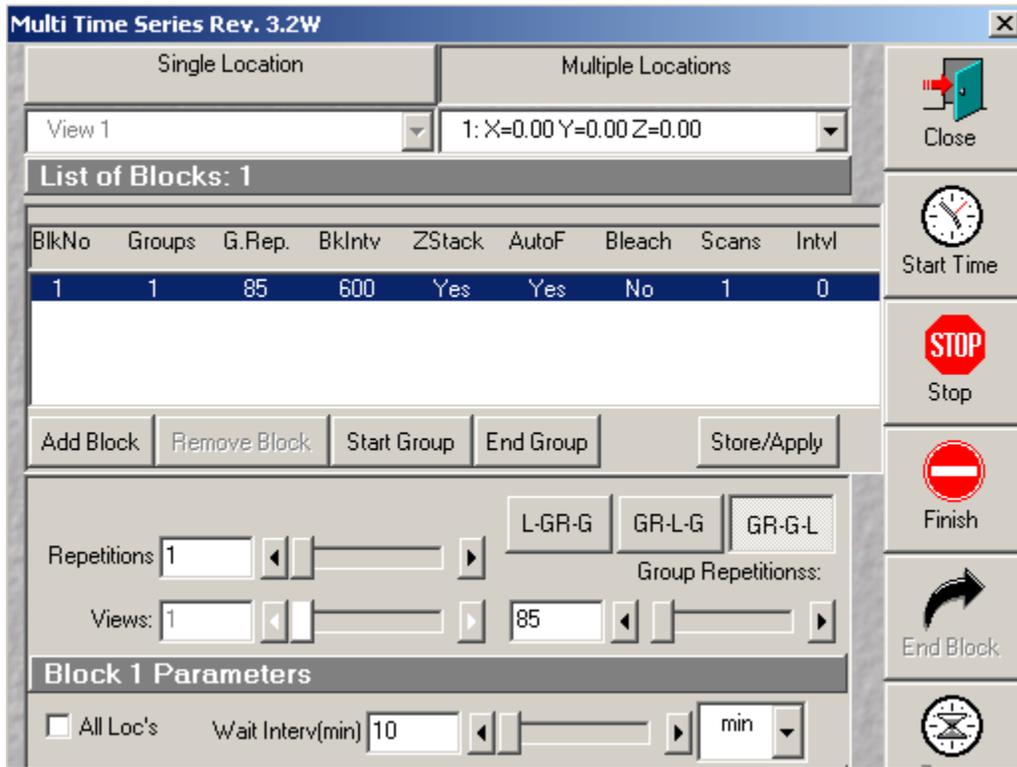
24. Go back to the top of the macro window and select Multiple Location. Check for different locations the autofocus settings and the multi track configuration. If you want to do an autofocus only on the first position, uncheck the 'Autof(XY)' option for the other positions!
25. Now you can start the macro with the Start Time button.

Some extra information

If you want to stop the macro you can do this with either Stop or Finish. Finish will stop scanning, but will concatenate all files that were already saved. Stop will stop the macro without concatenation . When the autofocus routine is active the systems will not be very responsive, you can better stop the macro during normal scans or during a pause.

With a scan speed of 1.5 sec./image and 6 optical planes you can follow 12 locations within 10 minutes.

Don't use the Options or Edit Location buttons. By changing settings here you can spoil experiments of the users after you.

Settings for List of Blocks:

You can add another block ('Add Block') with different settings e.g.:

you want to record your cells with a smaller time-interval the first 2 hours then later on. Block No 1 can have a time–interval of 5 minutes with 24 Group repetitions and block No 2 can have a time interval of 30 minutes with 20 Group repetitions for the next 10 hours. Be sure you check GR-G-L and make sure the other settings are the same as block No 1.

This section will be expanded in the future.

24-09-2004, Gert van Cappellen – Alex Nigg