Practical work no. 6: Live Cell Imaging
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Background

Live Cell Imaging: Most of the time, samples for fluorescence microscopy are fixed and permeabilized to preserve cell structure and to allow fluorophore stains to enter the cell. The specimens are then mounted between an objective slide and a cover slip. Live cell imaging brings along a variety of benefits not possible with fixed samples: observation of dynamic changes, reliable 3D structures etc.

Live cell imaging sets certain criteria also for the equipment used. To keep the sample alive during the experiment, cells need to be kept in growth medium, with CO_2 and temperature levels maintained at optimum, while on plates or chambered cover glasses. Inverted microscopes are mostly used just to be able to focus on the sample.

Time-lapse movies: Time-lapse movies are not done with video cameras in real time but as still images with a period of time between each image. Acquiring a specified number of still images with a set interval, e.g. 90 time points with 1 min between each image, results in an image set spanning 1½ h. If the interval between frames is now reduced to a frequency of 5 fps (frames per seconds), the changes too slow for human eye to capture can now be observed in an 18s time lapse movie.

Instrument and materials used

- Zeiss / Intelligent Imaging Innovations (3i) Stallion HSI live cell imaging workstation and SlideBook digital microscopy software (room B501b)
- Water pest (*Elodea*)
- Objective slide and a cover slip

Short description of the work

In this study, cells of a living plant (water pest, *Elodea*) are imaged. Changes in the live sample are observed by obtaining a series of images and presenting the results as a time-lapse movie.

Sample: In water pest cells, chlorophyll and other photosynthetic pigments in chloroplasts exhibit autofluorescence. Chloroplasts are in rapid movement due to extensive cytoplastic streaming. To best capture this movement, sample is kept in light before imaging. If the movement has decreased significantly, it can be reactivated by light exposure.

Leaf of a water pest is placed under a cover slip and the movement of the chloroplasts is captured as a time-lapse movie. Movement is at best very fast, i.e. easily observed through oculars. Exposure times for each images should therefore be kept well under 100 ms. Time-lapse interval (the time between individual exposures) should be kept under 1 s to ensure smooth transition between frames in the final movie.

How the results should be presented: Time-lapse movie is exported in a QuickTime movie format that is probably the best option for presenting video files in both PC and Mac computers.

Work instructions

1. Take a green leaf from a water pest and put it between a cover slip and an objective slide. Use a small amount of water to hold the cover slip in place and to keep the plant leaf from drying.

2. Place the objective slide in the heating stage's sample holder. *Note that neither CO_2 nor heating is needed in this experiment as water pest is an aquatic plant.*

Figure 1. Toolbar

3. Open FOCUS WINDOW by clicking the blue toolbar icon highlighted in Fig. 1. Using this window (Fig. 2) set FILTER CONFIGURATIONS as LIVE, and click GFP button to set it as a filter to be used (Fig. 2).

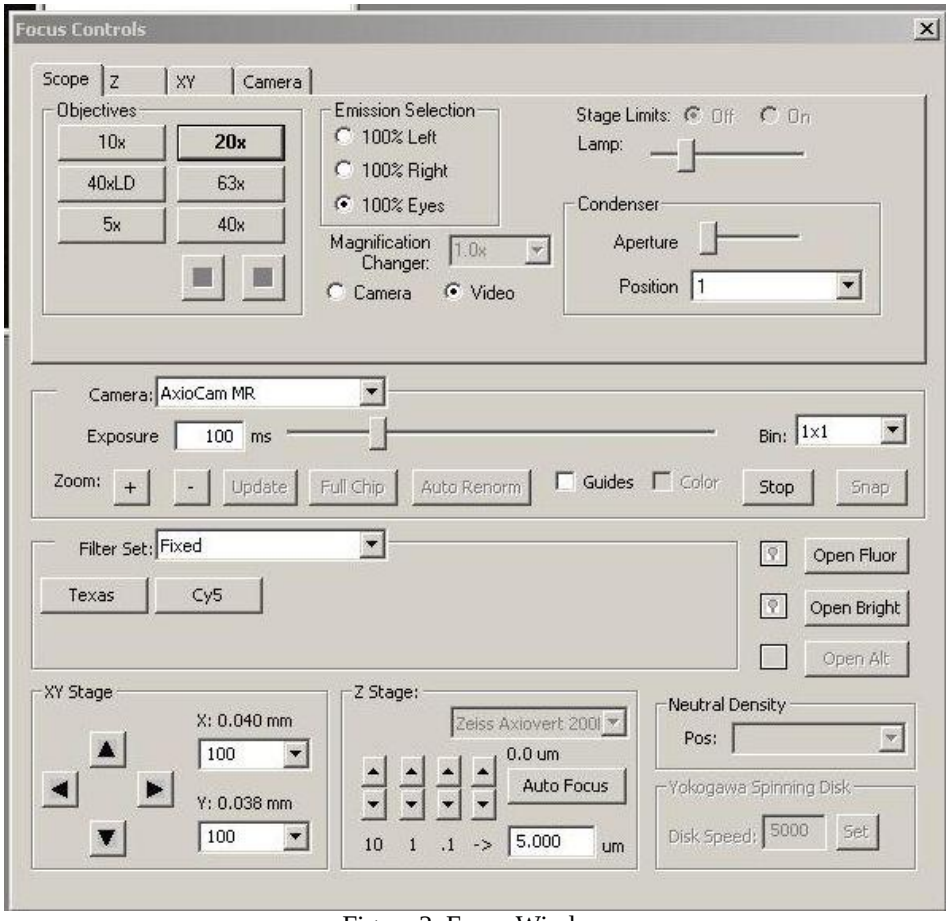

Figure 2. Focus Window

4. The 40x objective is used. Open fluorescence shutter by clicking OPEN FLUOR button in the FOCUS WINDOW (Fig. 2).

5. Use the joystick to move across the objective slide until you see a good spot through the oculars (leaf edges are the best places = thinnest). There is a “turbo button” at the end of the joystick to move the stage faster. Focus on the sample with the focusing wheels.

6. Change emission selection to 100% right (light is now diverted to the camera) and set the exposure time right in FOCUS WINDOW by moving the slide bar (Fig. 2). Keep shutter closed whenever not needed to prevent unnecessary bleaching of the sample by clicking CLOSE FLUOR button (OPEN FLUOR button has changed to CLOSE FLUOR) (Fig. 2).

Figure 3. Toolbar

7. Open CAPTURE window from main menu bar (Fig. 3) and set the following parameters (Fig. 4):

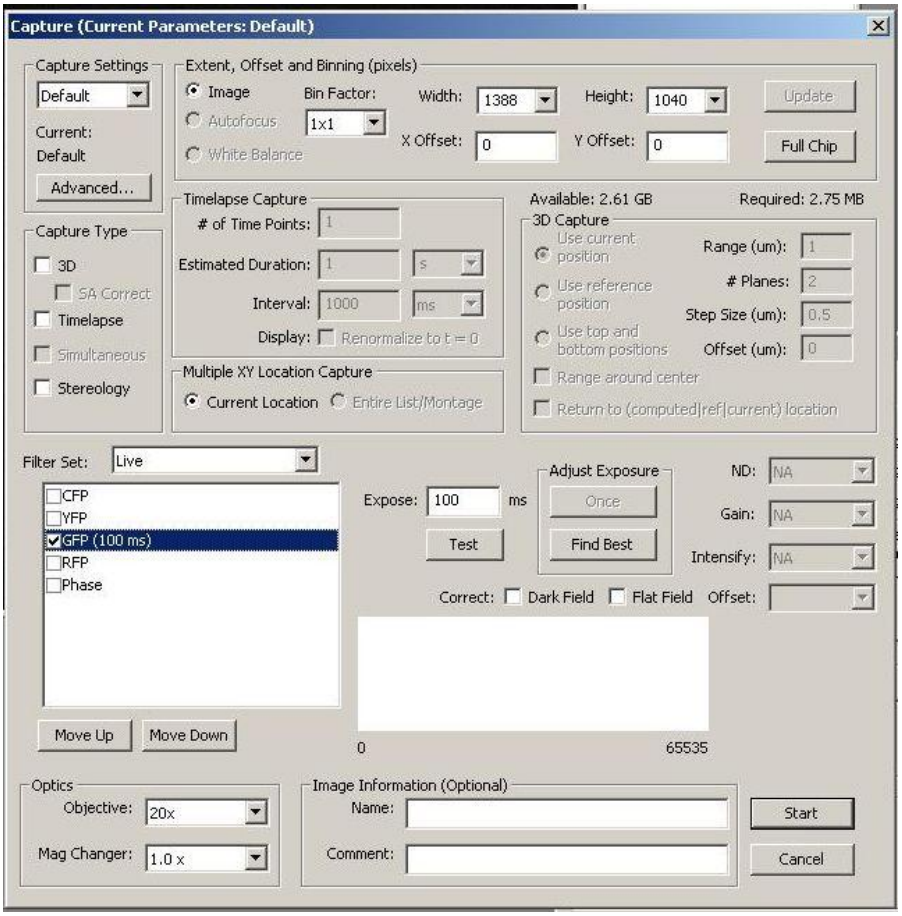

Figure 4. Capture window

a. Select TIMELAPSE
b. # TIME POINTS = 50
c. INTERVAL and UNITS = 500-1000ms. Experiment with different values, depending on the chloroplast movement. Too long intervals may not result in smooth movement in the final video.
d. Select CURRENT LOCATION: there is also a possibility to visit several predetermined points when choosing ENTIRE LIST (this is not covered in this work)

e. FILTER SET = LIVE
f. Select GFP tab and enter exposure time used. Check exposure clicking TEST button. Little or no red pixels (overexposed areas) should be seen.

8. Press START button to start image acquisition.

9. After acquisition the images can be saved as tiffs, .sld (Slidebook's own file format), or exported as a variety of movie files. This time they will be exported as a QuickTime movie.

10. Choose VIEW > CREATE SERIES MOVIE. Image window has to be active, else the option remains inactive.

11. Try e.g. 0.4 s as Frame Duration value and click OK. This way, if you have 50 time points, total length of the movie will be 20 s. You can experiment with different values and see how they affect the end result.

12. Select Movie export options: Apple quicktime and Lossless and choose location to save the file.

13. Open your movie file and adjust the frame duration if set too long. You can also capture another image set if you are not satisfied with the time point interval or exposure values. Copy the file also to your USB stick.

Quality of the movie is sufficient to be used in PowerPoint presentations etc. For image analysis, images should be exported as tiff series (Image > EXPORT > Channel Intensities…) and further analyzed with e.g. ImageJ.

14. End your session by taking out the sample.