

Mark & Find and Tile Scan

Motorized Stage Applications

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Motorized Stage Applications – Mark & Find and Tile Scan

Mark & Find and Tile Scan are part of the basic Leica Application Suite for Advanced Fluorescence. When using a motorized stage, both modules are activated to give full advantage of a high precision stage over a manual stage. Mark & Find enables the user to define multi position experiments to observe a large number of cells within one experiment, saving precious time. The Tile Scan feature allows the acquisition of overview images of the specimen with multiple channels and in three dimensions.

Mark & Find and Tile Scan are available on both the Leica AF6000 system series for widefield imaging and analysis and the Leica TCS SP5 confocal systems.

Mark & Find

High precision positioning is even more important when working with live cells. Preparing a live cell experiment not only takes time but also occupies the resources of the laboratory such as incubators, clean benches, etc. The experiment itself might run over hours or even several days. During the course of the experiment the acquisition system is out of use for other experiments. In the worst case scenario, the cells under observation do not show the desired behavior or do not react to the reagents added during the course of the experiment and the experiment has to be repeated. Precious time and resources are lost.

Such situations can be avoided with the Leica Mark & Find feature in combination with a high precision stage. These stages allow very precise repositioning of previously defined locations. Multiple positions, for example multiple cells in the same Petri dish, can be stored in advance of performing the experiment.

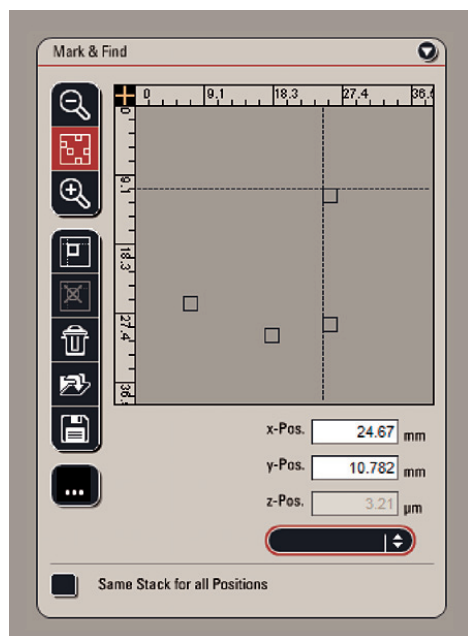


Fig. 1: Mark & Find interface: Perform multi positioning experiments and define location specific stack sizes.

How it works

With the Mark & Find tool the user can define the current position of the sample and recall it later on from a list together with other previously stored positions. This enables the user to quickly step through several positions to compare them or record multiple time series in a pseudo-parallel manner.

The user observes the live image and moves the stage to an interesting cell or location. Once the **mark** button is clicked, the x, y and z values of this position is stored. This procedure can be repeated for an unlimited number of additional positions. At each stage position a cell specific z stack size can be defined. Mark & Find can be combined with different contrasting methods such as fluorescence and DIC and with other experimental set-ups, for example, time-lapse.

Powerful in live cell imaging

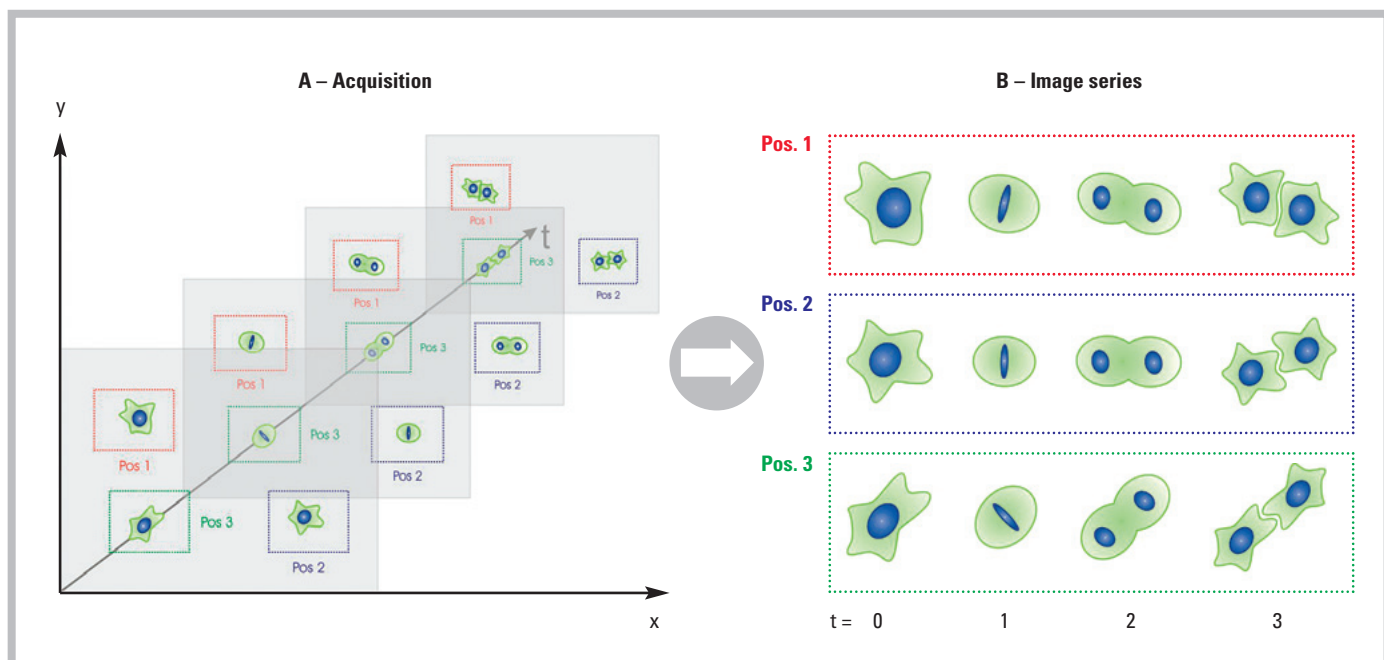
A simple, yet powerful application in live cell imaging is to mark miscellaneous positions of positive transfectants while searching for the most appropriate location. This helps users in the time-consuming process of screening through their sample, as they can always go back to a previous location if it is the right one.

Record multiple time series simultaneously

Mark & Find is particularly useful for studying dynamic processes in live cells. In many cases one needs to block and induce the biological process of interest by adding and removing drugs or replacing incubation media. The difficulty is then to quickly find a typical cell which displays the expected behavior during the course of the experiment.

Fig. 2: Capture all cell dynamics by recording multiple image series in a single experiment.

- A) Three positions have been marked prior to initiation of the experiment.
- B) Multiple movies can be recorded in a quasi-parallel manner and compared in real-time or stored for off-line quantification.



In such a case, Mark & Find is very useful, because the user is able to mark multiple cells previous to induction (Fig.2 A). By recording a time series in conjunction with Mark & Find, it is now possible to record multiple cells in a quasi-parallel manner (Fig. 2 B). The chances of observing a process of interest in one single experiment are much higher compared to the conventional situation where only one cell can be observed at a time.

In effect, Mark & Find can help the user to save time or observe a larger number of cells. If the aim is to obtain quantitative parameters from the experiment (i.e. size, migration speed, particle tracking) a gain in throughput will effectively improve the statistical significance of the data.

Mark & Find can be applied over several rounds of cell cycles, too. Additionally, multiple cells can be observed and their behavior can be compared.

Applications in developmental biology

Since Mark & Find can be combined with both t-series and z-scans, very complex recording scenarios become possible such as following dynamic processes in large specimen at multiple positions over time, each with an independent z-stack. Applications in developmental biology are perfectly suited to such tools, for example to follow the development of the nervous system and transport systems of animal models.

Figure 3 shows a real-world example of a time-lapse recorded using Mark & Find. NIH 3T3 mouse fibroblast cells expressing H2A-YFP stably and were incubated with Aphidicolin over night. This leads to an arrest in the G1-/S-phase transition. The shown time series was observed 4.5 hours after removal of the blocking agent. The cells were monitored for another 4 hours. In total 10 positions were selected using Mark & Find over time, and z-stacks of 7-8 μm with a z-step size of 0.3 μm were recorded for each cell. Out of those ten cells, only one could be followed through mitosis (left column), whilst the others did not divide during the observation time (right column). This is a typical situation in live cell imaging when biological processes are triggered using specific drugs. Mark & Find increases the chances of observing such a process in a single experiment. DIC (differential interference contrast) and fluorescence images of the cells are shown over time from the top to the bottom. All DIC images are single in focus slices of their respective z-stacks, H2A-YFP images shown as maximum projections over the whole stack after deconvolution. Magnification 63x objective.

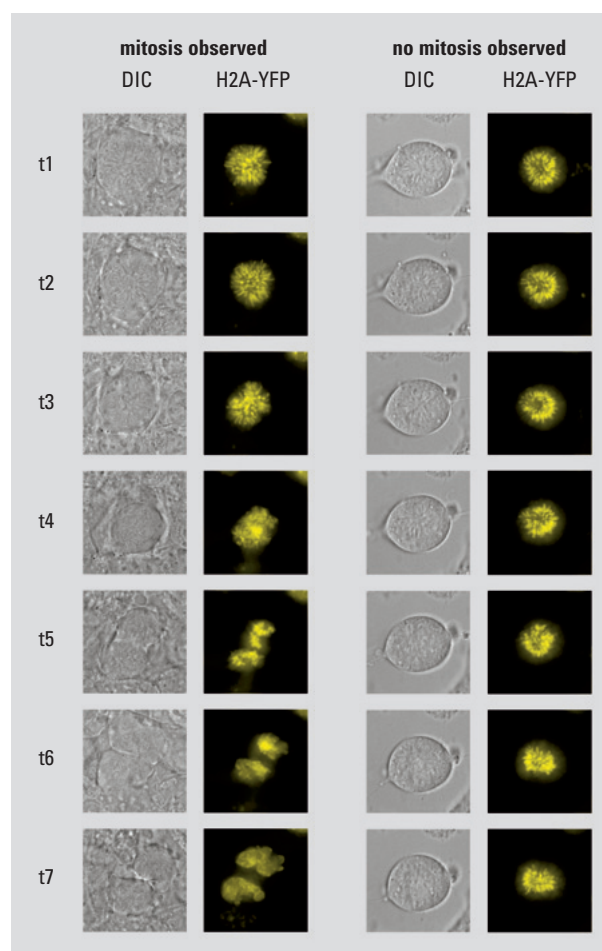


Fig 3: NIH 3T3 mouse fibroblast cells expressing H2A-YFP. Two positions of a Mark & Find experiment. Courtesy of Constantin Kappel, DKFZ, Heidelberg, Germany

Tile Scan

The Tile Scan module allows the user to automatically assemble an image from multiple smaller images. Large specimens that are bigger than the current field of view can be viewed as a whole using the Tile Scan function. This works by taking a number of adjoining images of the sample (the "tiles") and merging them digitally to a larger view of the scene.

How it works

A tile scan area can be defined by marking just two opposite positions defining the outer points of the area to be scanned. The positions are marked in the same way as locations in Mark & Find. Depending on the field of view, the Tile Scan feature will calculate the number of tiles needed to cover the scan field.

Produce high resolution overview images

Figure 5 shows reconstructed *Drosophila* larval salivary glands taken with a 20x objective lens. The user is presented with the option of preserving the original images as well as the overview image for reference. Since the automated stage under full software control, the user only needs to specify the boundaries of the area of interest, the software will automatically calculate the area to be scanned.

Find live transfectants fast

In the live cell imaging of transiently transfected cells, one often faces the problem of finding a cell expressing the protein of interest at the correct level. With Tile Scan it is now possible to produce a "bird's eye view" image of the entire coverslip or culture dish to aid the user in finding interesting parts of the sample more quickly. Such an overview image can serve as a reference for the whole experiment and can also be used to obtain important quality parameters, for instance cell density, transfection efficiency or cell viability. An added advantage is that the cell, which was captured in more detail, can still be viewed in its context later on.

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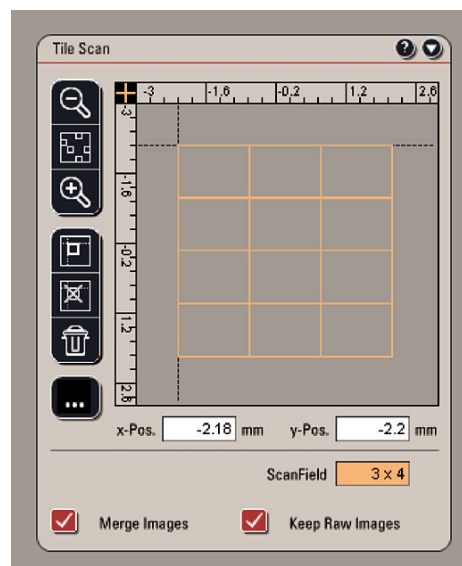


Fig. 4: Tile Scan interface: Get an overview image of your specimen with multiple channels and combine it with z-stacks.

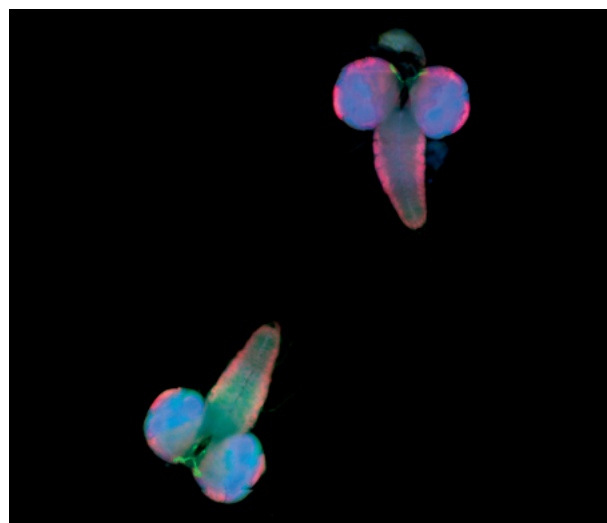
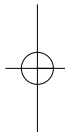
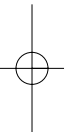
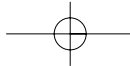


Fig. 5: *Drosophila* salivary glands at 20x magnification with bright field and fluorescence channels overlaid. The whole image was assembled from 5x3 single images at full resolution (1392x1040 per image). Image shows nuclei (DAPI) in blue, Neurons/Axons (Alexa 488/Cy3) in green and neuronal nuclei (Alexa 594) in red. Note; some crosstalk between Cy3 and Alexa 594 is visible, as the image was captured with a broad band pass BGR cube. The merged image is completely unprocessed. Courtesy of Dr. Christoph Melcher, Research Center Karlsruhe, Karlsruhe, Germany



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