

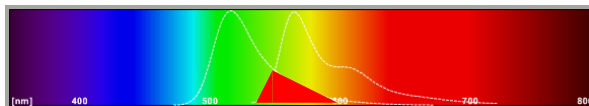
Sequential Scanning in LAS AF.

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Sequential scanning is an advantageous tool when detecting multiple fluorophores in one sample. It will enhance the image quality (e.g. avoiding crosstalk) by recording in a sequential order instead of a simultaneous acquisition. This application note is designed to give you a step by step to setup a sequential scan using LAS AF.

When to use sequential scanning

When two or more fluorochromes are simultaneously excited in a specimen, it cannot be avoided that several fluorescence signals are recorded in a detection channel and can no longer be separated in the images. This crosstalk (also called bleedthrough) can be avoided by using the sequential imaging recording method.

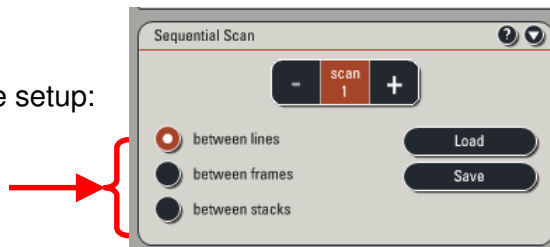


Another advantage of using this approach is when the confocal microscope system is equipped with less detection channels than needed. Hence, it is possible to do a triple (or more) detection with a microscope system that has only 2 detection channels.

Optional sequential modes

Three different modes of sequential image can be setup:

- between lines
- between frames
- between stacks

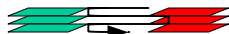


“between lines”



In this mode, the detection channel and the excitation wavelength are changed after the scanning of a single line, going from one to the other fluorophore settings. This pattern is repeated until a full image is completed. This mode is particularly useful to minimize movement artifacts occurring in *in vivo* experiments. Also, this mode is especially suitable for any time-dependent measurements.

“between frames”



In this mode, first a complete image using the first fluorophore instrument parameter settings (scan 1) is performed. The system then switches to the second fluorophore settings (scan 2) to record the same optical section. This mode allows image recording even using UV or MP lasers. In addition, this mode provides the possibility to record a triple stained sample with a 2-channels microscope system, since there is sufficient time between frame to change critical hardware parameters, which is not the case using the “between line” mode.

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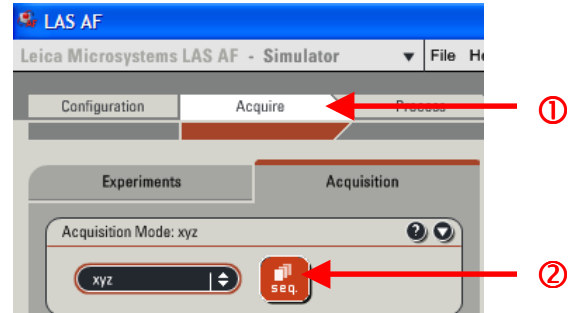
“between stacks”



In this mode, all the optical sections from the same image series are recorded using the same instrument parameter setting (scan 1) before the system switches to the next sequential instrument parameter setting (scan 2). This method is suitable for immobile or fixed samples. In addition, fluorescent dyes prone to bleaching can be recorded in a less damaging way in this mode.

PROTOCOL

1. In the **Acquisition** window ①, Click on the **Sequential** button ②. The Sequential window will automatically drop down ③.



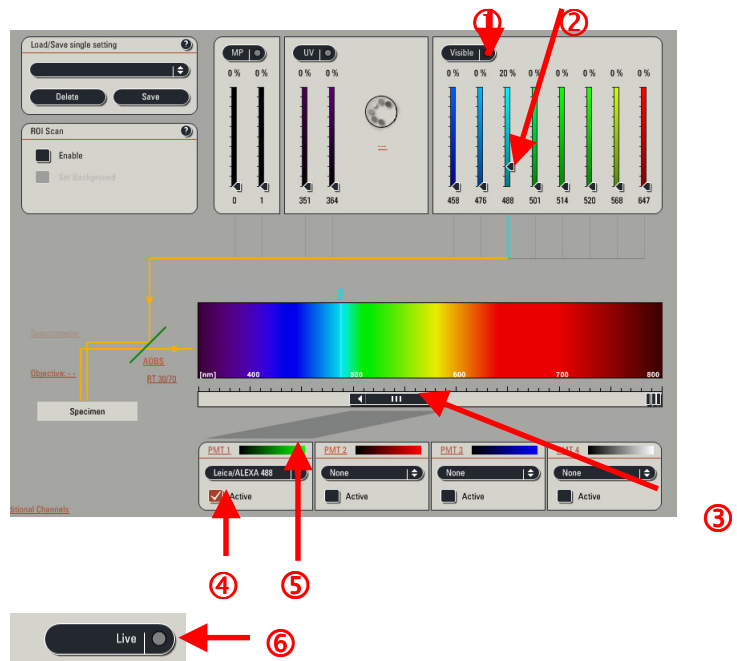
2. The sequential scan can then be set up:

- **Scan 1** is automatically selected ④.
- You can choose to scan **between lines**, **between frames**, or **between stacks** ⑤.
- You can **Save** your laser beam path settings or **Load** a previously saved configuration ⑥.



3. **Scan 1** configuration:



- Activate the visible laser ①.
- Set up the laser power ②.
- Place your PMT in relation with the expected fluorophore spectrum emission (in our example Alexa 488) ③.
- Activate the PMT ④.
- Select the fluorophore color ⑤.
- Activate the **Live** mode to adjust your settings ⑥.

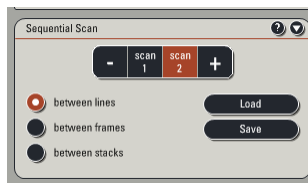



Confocal Application Notes

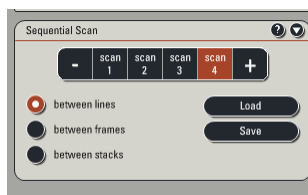
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4. Scan 2 configuration:

- Click on  button. The  button will appear.
- Setup the laser configuration for your second fluorophore as described for the Scan 1 above.



If more fluorophores are present in the sample, then click on  button again. (PS: A maximum of 4 sequential scan can be performed using between line mode acquisition.)



5. Acquisition:

- Click on the **Start** button to initiate the sequential acquisition.

