# Calibration

# Frame scan mode

# Point jump mode

point 2

parked

point 1

point 2

start

fly-in

galvo & pixel sampling

point 1

stimulation

delay

stimulation

duration

hold

jump

parked & jump

point 2

After an image is acquired in the *frame scan mode*, a series of point ROIs can be added to the image which can be used in the *point jump mode*. In point jump mode, both optical recording of fluorescence signals from the point ROIs and stimulation of these points is possible either separately or combined.

The following parameters describe the point jump mode:

* **Record**

If active, optical recording of the fluorescence signal at each point ROI is done during the entire holding time. To activate this option, there must be at least one fluorescence channel configured to receive pixel data.

* **Moving average filter**

Applies an N point moving average filter to all ROI fluorescence signals if recordings are activated during the holding time. The holding time is adjusted to be a multiple of the N-points used for the filter.

* **Average all**

If active, all fluorescence recorded within the hold time is averaged to a single data point.

* **Start delay**

Initial delay from the global start trigger to the start of the first point jump sequence defined to be the beginning of the galvo hold time at the first point ROI in the first sequence. This initial delay is composed of a delay during which the galvos are kept at their parked position and a jump time needed to position the galvos to the first point ROI.

* **Hold time**

Time during which the galvos are stationary at a certain point ROI.

* **Stimulation delay**

Delay measured from the moment the galvos are stationary at the point ROI to the moment a stimulation is initiated. This delay together with the stimulation duration is less than or equal to the holding time.

* **Stimulation duration**

Time during which a stimulation takes place at the given ROI. This time together with the stimulation delay is less than or equal to the holding time.

* **Point jump time**

Time during which galvos are repositioned to a new ROI which includes a minimum jump time determined by the galvo calibration procedure and optionally, a time during which the galvos are returned to their parked position, kept there and jumped to the next ROI. The *point jump time* can be modified if the option *parked after each point* is activated, in which case, the minimum jump time will be equal to the jump time to the parked position and from the parked position to the next ROI. The ROI jump time is equal to the largest ROI jump time of the two galvos.

* **Parked at sequence end**

At the end of visiting a sequence of point ROIs, the galvos are returned to their parked position and a new sequence is initiated from this parked position. If galvos are not parked at the end of a ROI jump sequence, they will jump to the first point ROI in the sequence. If this option is selected then the *sequence period* may be freely adjustable as long as it is greater or equal to the minimum sequence period in which case, the galvos will remain at their parked position during the additional time.

* **Parked after each point**

The galvos are returned to their parked position after each ROI and it allows to modify the *ROI jump time*. In this case, the time spent in the parked position minus the time to jump there and then to the next ROI is equal to the time the galvos remain in their parked position.

* **Sequence period**

Time from the start of the first point ROI holding from a previous jump sequence to the next jump sequence. If the option *parked at sequence end* is not active, then this value cannot be modified and is equal to the *sequence duration* plus *ROI jump time*. If the option *parked at sequence end* is active, then an arbitrary sequence period can be chosen that is larger or equal to the minimum sequence period calculated when the option *parked at sequence end* is not active.

* **Repeat sequence**

Number of times to repeat a point jump sequence.

* **Sequence duration**

Time to complete ROI point jumps which does not include the jump time from the last ROI to the first ROI in a next sequence or from the last ROI to the parked position.

* **Set all ROIs**

If this option is active, the current point jump settings are applied to all ROIs.

* **ROI list**

Displays a list with user selected ROIs for a given image. Each ROI can be activated/deactivated by a tick mark to the left of each ROI name.