Topic

This technical note describes the steps required to ensure maximum frame rates are achieved from the combination of hardware making up the UltraVIEW VoX spinning disk confocal system.

Discussion

The UltraView VoX system is made up of components selected or designed by PerkinElmer to be fast, ideal for live cell imaging, however these components must be used carefully in combination to achieve high speed imaging.

Computer Settings

Anti-virus scanners that have a "On-access" or "Live" scanning mode can seriously degrade acquisition performance in Volocity. Ensure that "On-access" scanning is disabled for the whole computer, or for Volocity and Volocity libraries.

Fast Sequential

To minimise the hardware movements between channels, set the UltraVIEW VoX to Fast Sequential mode.

Light Paths

Light paths store the hardware configurations necessary to image the different fluorochromes or dyes present in the sample. When preparing for the experiment, identify the device settings that **have** to be changed to switch between fluorochromes. Attempt to minimise these changes then store only these devices in the light path for each fluorochrome.

Do not store a device if it does not change between fluorochromes. The software will then not send a command or poll a device needlessly.

Do not store the 'Sensitivity' camera setting if present as this can take some time to change.

In an experiment that uses only fluorescence illumination leave all other shutters set to 'Don't change' rather than 'Closed'. Even if a shutter is already closed a light path which includes the 'Closed' state may send a command to the shutter. When the shutter state is 'Don't change' no command is sent.

In summary:

- · Store the bare minimum in light paths
- · Never save the 'Sensitivity' camera control
- · Set shutters to 'Don't change' if they are not required by the experiment

Camera Settings

Changing exposure times between channels does not incur a significant penalty for a fully synchronised UltraVIEW system.

Use live enhancement tools to ensure exposures are not unnecessarily long. Live enhancement will confirm that low signal intensity levels are being detected by adjusting the display look up table.

Freeze the live preview during acquisition so that resources are not being shared with the preview to improve timepoint start accuracy.

In summary:

- Use shortest exposure times possible
- Freeze the live preview during acquisition

High Speed Acquisition Checklist

To get the highest frame rate from your UltraVIEW system, follow these guidelines:

- Disable "On Access" or "Live" virus scanning
- Set the UltraVIEW VoX to Fast Sequential mode
- Store the bare minimum in light paths
- Never change the 'Sensitivity' camera control between channels
- Set shutters to 'Don't change' if they are not required by the experiment
- Use shortest exposure times possible
- Freeze live preview before starting acquisition