System initialization

“GeneralPurposeSOS.vi” is the main VI of the program. All the user operations will be performed through this VI. When start running the program, a popup window will appear and allow the user to choose which camera to turn on, depends on the type of experiment the user is planning to do. Check “Wide-Field Imaging Camera” and click “OK” if corresponding type of detection mode is to be used, and the same to “Spectrograph Camera”. Click “use both” if both detection modes will be used.

During the camera initialization process, one can see the “Camera On” LED of the selected camera is on, and a blinking LED on the front panel, with the label “Initializing”. After a couple of seconds, it will stop blinking and becomes “Ready”.

Parameter settings

All the parameters can be entered before running the program, or in the idle state when the program is running.

1. System Settings:

Include data saving path, experimental name, illumination mode and detection mode. The obtained data will be saved with the input in “experimental name”. Note that if only one camera is selected for using at the beginning, some of the items associated with the other camera will be disabled for easier using.

2. Camera Settings: (items are the same for both cameras)

Camera T(°C): cooling temperature of detector.

Cooling On: One can click the “Cooling On” to start cooling down the detector. The “Cooling” LED will then be switched on and one can monitor the cooling process in “Current T(°C)” indicator in the camera status panel. The “Cooling” LED will switch off when the detector has stabilized at the input temperature. If the “Cooling On” is not clicked, the cooling will be skipped.

Camera DAQ mode: “Live Monitoring” allows one to visualize the image for arbitrary amount of time, under specified exposure time. One can save the currently displayed image (after pressing “Stop Acquisition”) by clicking “Save Image” button (will save only one frame). “Kinetic Series Scan” allows one to take a video data file with preset number of frames. The current frame index is updated in “Current Frame” indicator in the camera status panel. The “Save Image” button is disabled since the video data file is saved by default in this mode.

Number of Frames to Acquire: number of image frames to be acquired in “Kinetic Series Scan” mode. This can be ignored in “Live Monitoring” mode.

3. TIRF-Confocal Settings:

In this mode, the instrument is programmed to take certain number of frames input in “Number of Frames to Acquire” in “Wide-Field Imaging Camera”, and then switch to confocal data acquisition mode to take a trajectory with length specified in “Trajectory Length”. These two sections together are considered as one TIRF-confocal trajectory. All the data are automatically indexed and saved by default in this mode.

Number of Traj to Acquire: number of TIRF-confocal trajectories to be acquired. The program will keep taking data until specified number of trajectories has been taken. The index of current trajectory is shown in the “Current Traj#” indicator in camera status panel.

Spot choosing region: the region in the image in which a molecule will be picked to perform confocal mode. If the camera is coupled with a signal splitting system, one can choose whether the left or right part of image (by selecting “Donor” or “Acceptor”) is to be passed to pick molecules. Simply select “Entire image” if the whole image is to be used. The index of selected molecule and its coordinate will be displayed in the “Spot Index” and “Spot Center” in camera status panel.

Data acquisition

Start Acquisition: After all the parameters have been set, the “Start Acquisition” can be click to start taking data. The image view panel will automatically switch to the selected detection mode; and the “Ready” LED will become “Acquiring” and start blinking until the acquisition is complete or stopped.

Stop Acquisition: One can stop the acquisition by pressing “Stop Acquisition” at any time in each data acquisition mode. The “Acquiring” LED will change back to “Ready” and stop blinking. The instrument is now in idle state and ready to take new data.

Save Image: In certain detection mode (Live Monitoring in both wide-field imaging and imaging spectrograph), this button is enabled and is used to save the currently displayed image (one frame). A popup window will appear for selecting image saving path and name.

Skip Current Traj: In “TIRF-Coupled-Confocal” panel, this button can be clicked to manually skip the current trajectory.

Note that one can start acquisition before the cooling process finishes. Note also that the “Current T(°C)” indicator will stop updating in both camera status panels during data acquisition, and will restart to update in idle state.

Camera Status

Include status of cooling, temperature, index of frame, trajectory, and spot mentioned above. “Flip mirror” allows one to monitor the position of each motorized flip mirror. Note that all the items in this panel serve as indicator, not for control.

System shut down

Quit Program: Stop the program without turning off the camera. The cooler will still be on and the detector will maintain its cooling temperature.

System Shut Down: Stop the program and turn off the camera.