Olympus FVMPE-RS Hybrid Multiphoton System
Standard Operation Protocol
Basic Operation

Turn on system

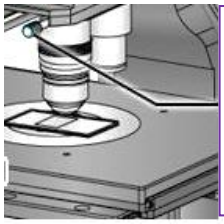
Please sign on the log sheet before switching on system.

- Turn on Chameleon laser by turning the Laser Key 90° clockwise to horizontal position (if only one laser needs to be used, please only turn on laser 1)
- Turn on computer and log in with "**User**"①
- Turn on power supply Unit FV30-PSU ② (please wait for about 10 sec before next step)
- Turn on power switch of Main scanner ③ (please wait for about 10 sec before next step)
- Turn on power switch of SIM scanner ④
- Turn on fluorescent lamp illuminator ⑤ (the switch is at the left back of the box, shown by →), wait for 3sec, press the "ON/OFF button at the front panel
- Turn on microscope power ⑥
- Turn on the touch panel controller ⑦ (the switch is at the right back of the box, shown by →)

Setting the microscope

- Double click the "FV30S-SW" icon 

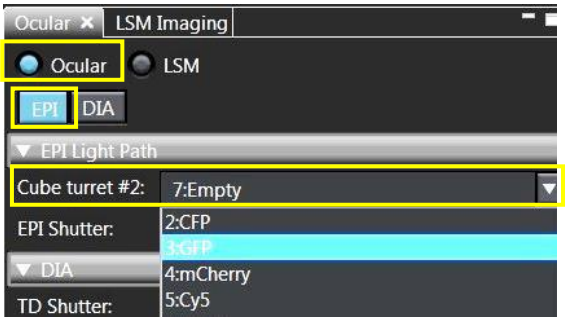
on the desktop to initialize the software, it may take 1-2min for software and hardware connection.
- Keep pressing  on touch screen to move the objective up
- Select the objective lens manually and engage it in the light path →
- Put your sample on the stage
- Select **Ocular** to set the microscope for direct observation via the eyepieces
- Click on **EPI** for fluorescence observation
- Select the filter for fluorescence observation in the drop down list of **Cube Turrent #2**.



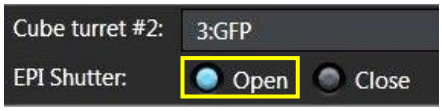
Pull the handle out or push it in to select objective

Objective go up

Objective go down

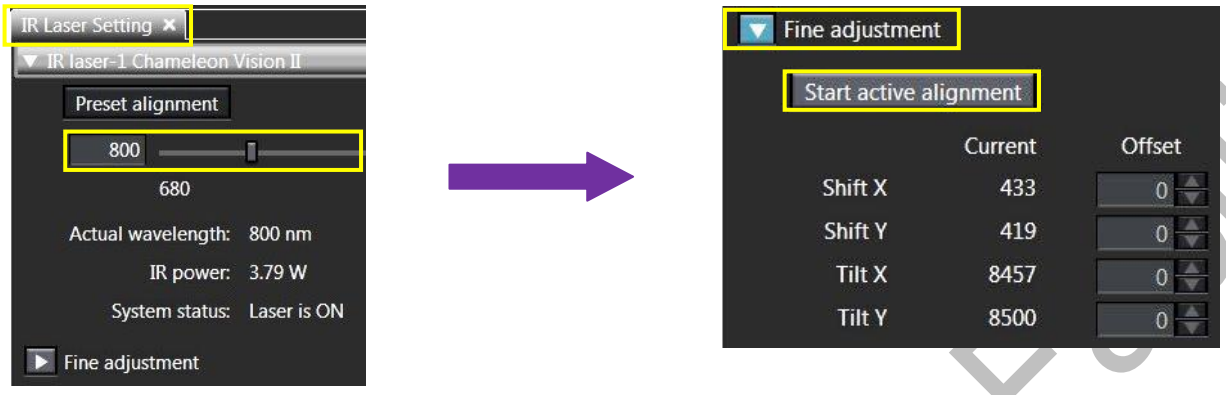


- Put water on the sample if water objective will be used.
- Keep pressing  on touch screen to move the objective close to the sample
- Click **Open** in **EPI shutter** to open shutter if necessary →
- Focus the sample with Corse/fine focus knob
- Locate the view of interest through stage control knob

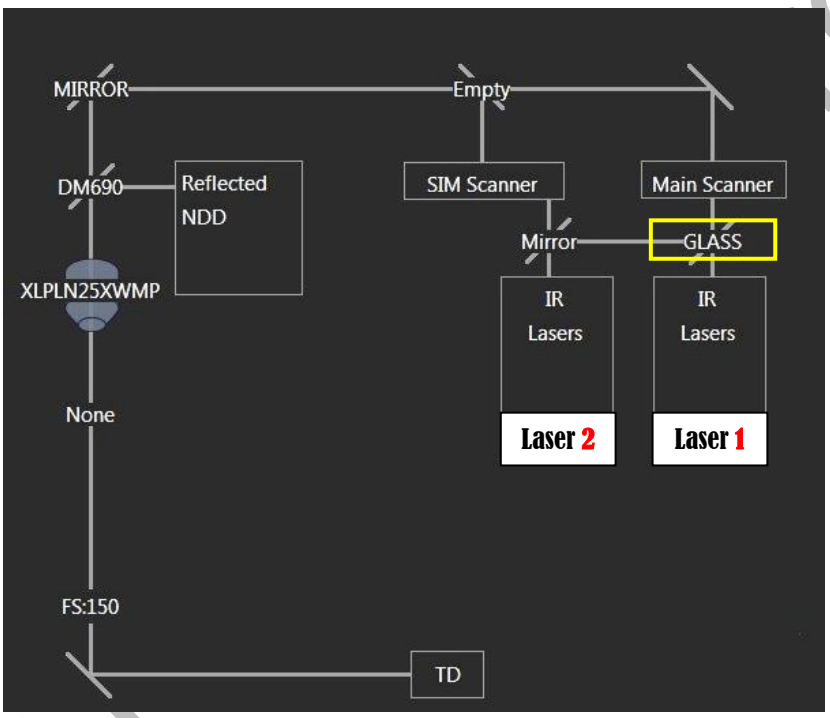


Configuring the beam path and laser

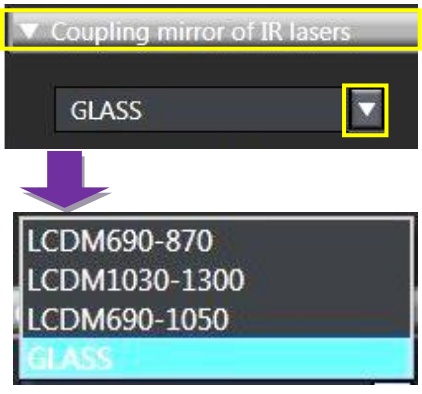
- In **IR Laser Setting** Tool Window, set the wavelength of the IR laser to be used.
- Press the button ▶ of **Fine adjustment**, and press the button **Start active alignment** which will align the laser to optimal position.



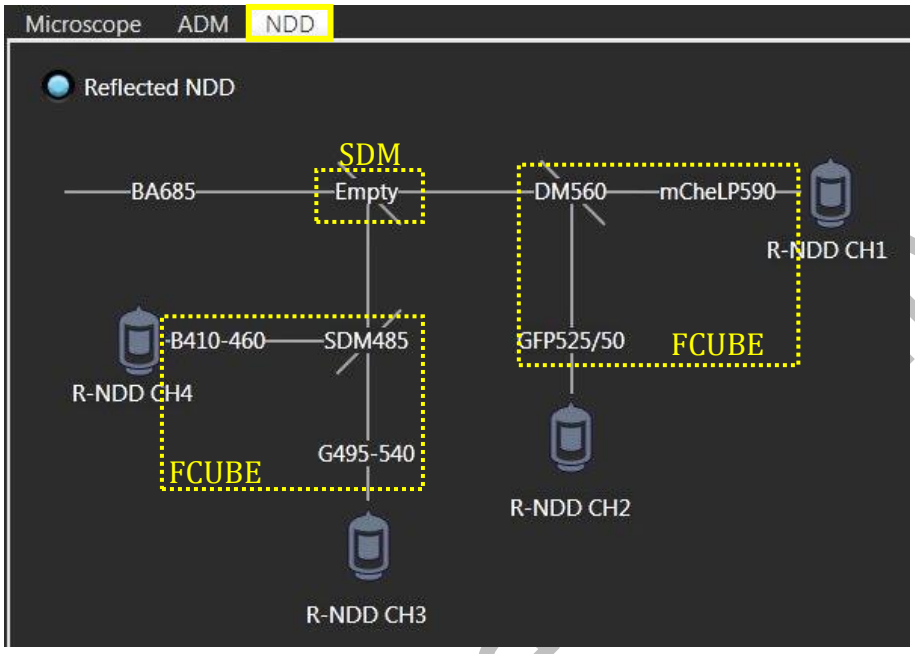
- Click on the **Lightpath** button to open the **Lightpath** window.



- Select coupling mirror in **Coupling mirror of IR laser** tool window depends on the wavelength to be used.
  - Chose "**Glass**" if only laser 1 is in use.
  - Chose "**LCDM 690-870**" for two laser lines with Laser 1 >=920nm and Laser 2<=870nm
  - Chose "**LCDM 1030-1300**" for two laser lines with Laser 1 <=940nm and Laser 2>=1030



- Check NDD filters in **NDD** panel of Lightpath



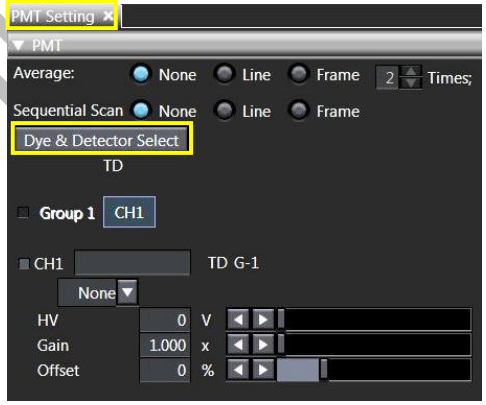
SDM	
SDM570	>570nm pass to Ch1/2, <570nm reflect to Ch3/4
Empty	All pass to Ch1/2
Mirror	All reflect to Ch3/4

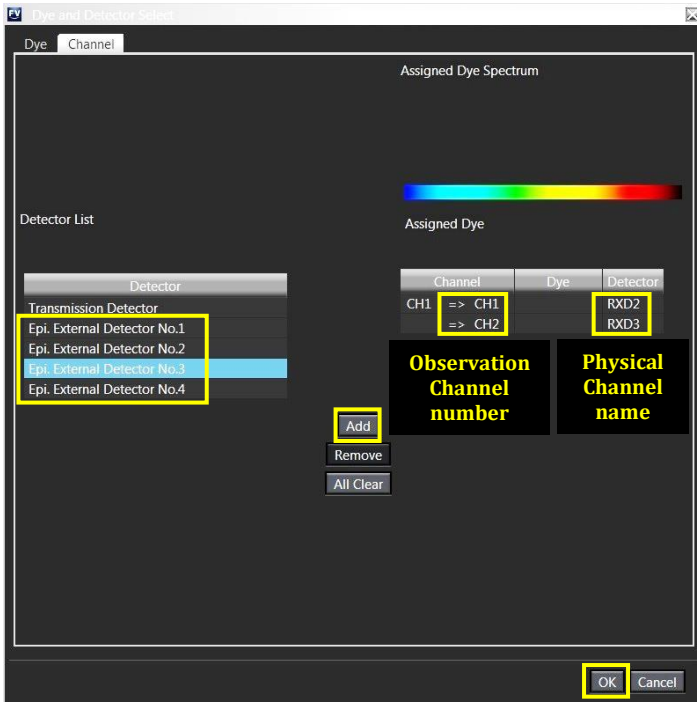
NDD filter cube list at FCF:

FCUBE	Dichroic mirror	BF for Ch1/Ch3	BF for Ch2/Ch4
Blue/Green	DM485	G 495-540 (Green)	B 410-460 (Blue)
Red/FarRed	DM650	FR 660-750 (FarRed)	R 575-645 (Red)
GFP/mCherry	DM560	mCherry LP590	GFP 525/50
mCherry/Qdot655	DM630	Qdot 655/40	mCherry 600/50
SHG/YFP	DM495	YFP 535/50	SHG 460/50

Please be noted that FCUBE filter might be changed based on user's application. Please contact FCF staff if the FCUBE filter in use does not fit your application.

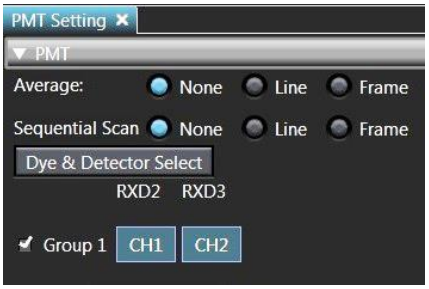
- Press the **Dye & Detector Select** button in **PMT Setting** tool window. The **Dye & Detector Select** dialog box appears
- Select the detector to be assigned to the observation channel in Detector list
- Press the **Add** button. The observation channel number and the abbreviation (physical channel name) of the assigned detector are displayed in the observation channel list.
- After setting all channels, press the button **OK.**



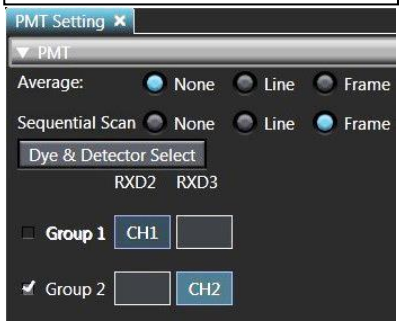


- If sequential scan needed for multi-channel acquisition, click **Line** or **Frame** in **PMT Setting** tool window

Simultaneous scan



Sequential scan

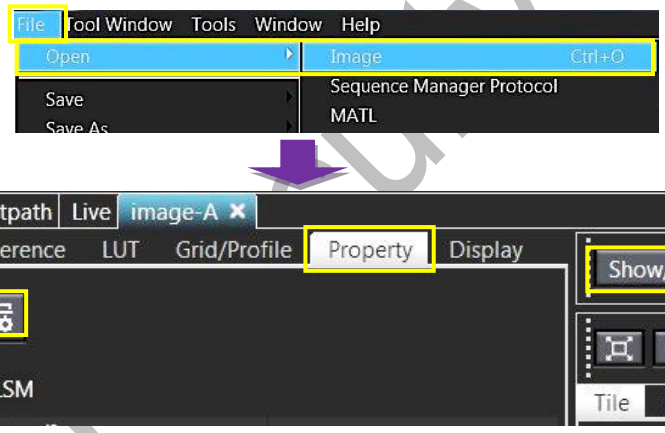


- **Simultaneous scan**
  - Use for one or two excitation wavelength
  - Advantage: faster image acquisition
  - Disadvantage: cross talk might be happened between channels

- **Sequential scan**
  - Only applicable for two different laser wavelength
  - Advantage: when one group is active, only one detector and one laser line is switched on. This may reduce cross talk
  - Disadvantage: slower image acquisition

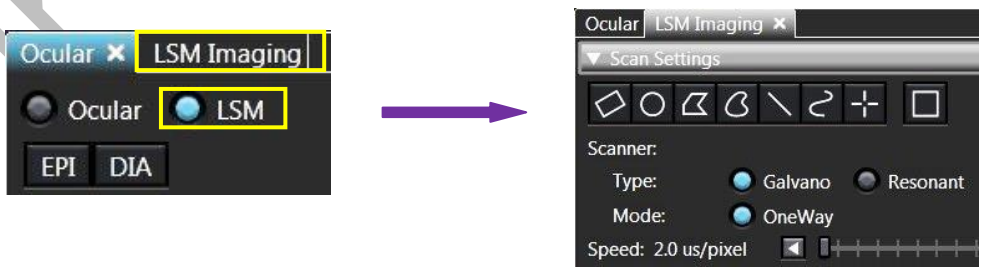
Reuse previous acquisition settings

- Select **Open** - **Image** in the **File** menu to display the dialog box, and select the image to view.
- Click **Show/Hide** and select **Property** to load information of acquisition settings
- Click  to reload previous setting

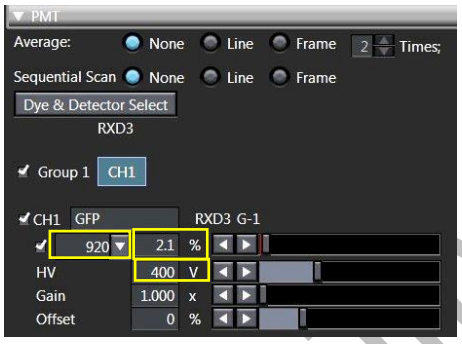


Adjusting the live image

- Click on the **LSM** button to switch to scanning mode, then click **LSM imaging** for scan settings



- In **PMT** window, select the laser, set an initial value for laser intensity and HV value
  It is suggested to set the laser intensity <5% and HV <800
- Press the Live button in the **Live** window. （The button view is changed to Stop .) The live image is displayed in display area.
- In **PMT** Window, fine tune Laser Intensity (%), Sensitivity (HV), Gain and Offset to optimize the image



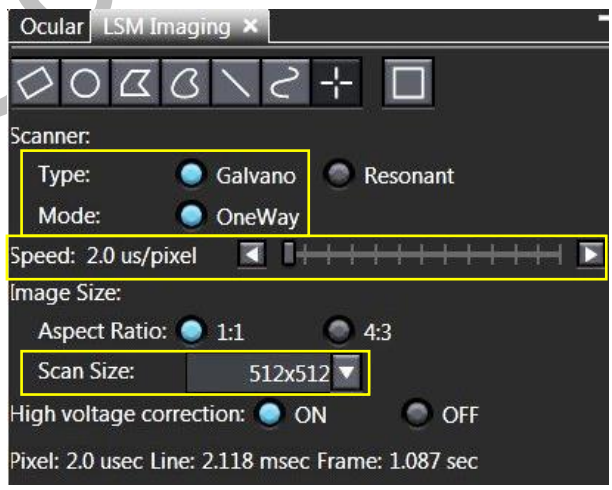
- Click on Hi-Lo button to display saturation pixels. Red = Saturation (maximum). Blue = Zero (minimum).
- Adjust **laser intensity (%)** and **HV** to obtain few red pixels shown on image. Increase **Offset** to introduce blue pixels in background area.
- Click Stop to complete the preview of the sample.

** User is advised **not to use too extreme settings** on **laser intensity** and **HV***
(as too high laser power will induce bleaching onto the sample; sample burning might be happened if the laser power is too high; and too high HV will cause significant noise and shorten the lifetime of the PMT).
*In any given cases, **users should keep the HV < 800**.*

Acquiring an image

- In **LSM Imaging** Tool Window, select the scanner **Type** and **Mode**.

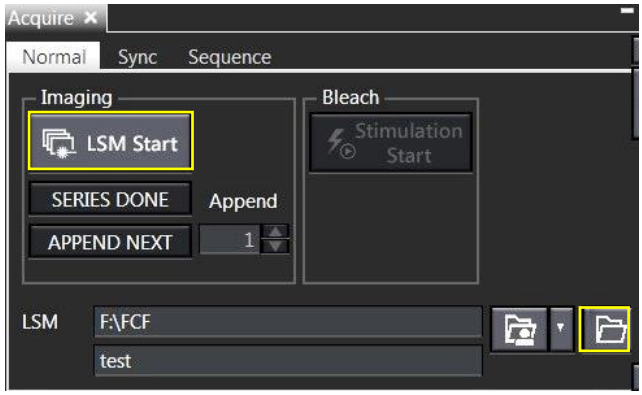
Type	"Galvano" (High definition scan) or "Resonant" (High speed scan)
Mode	"OneWay" (Scan in one direction) or "Roundtrip" (Scan in both directions)



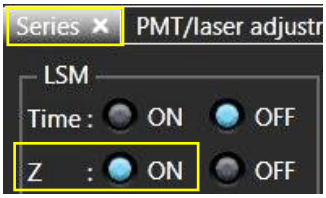
Galvano and **OneWay** are suggested in regular acquisition.

- Use the slider in the **Speed** to adjust the scan speed. Slower speed increases signal/noise ratio of the image.
- Select the **Scan Size** as predefined number of pixels.
- Select the number of **average** in **PMT** tool window. Averaging improves the image by increasing the signal-to-noise ratio.

- In "Acquire" tool window, press the button and select your folder in **F** drive to save the images. The acquired images are saved automatically.
- Press LSM Start to start acquiring the image.

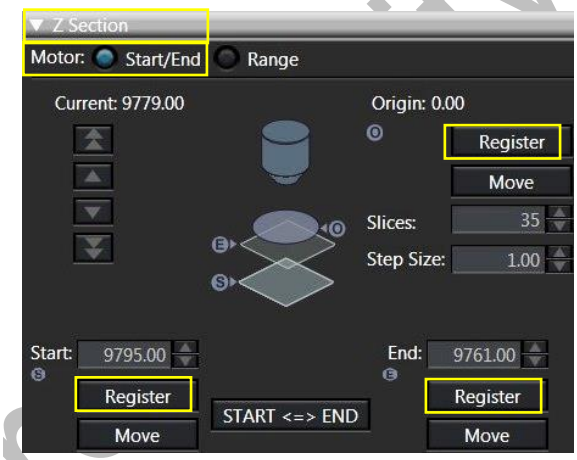


Scanning Z Series

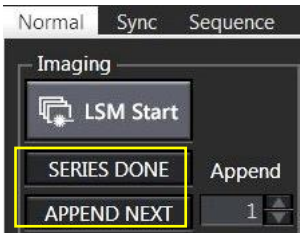


- Select **ON** for **Z** in **Series** Tool Window.
- Select "**Start/End**" in **Motor** on **Z Section**.
- Click Live button to have continuously scanning.
- Press the Register button in **Origin** at the Z position which is the reference position to acquire the images.

**The distance from the Z position registered in [Origin] to the current Z position is displayed in [Current].*
If you register the top surface of the specimen in [Origin], the depth from the top surface to the observation position is always displayed in [Current], which is very useful.

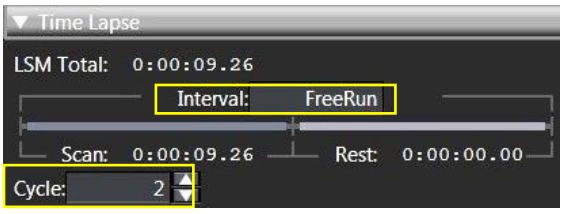


- Turn the focusing knob gently to locate the start position of the specimen to **Register** start**.** Then locate the end position of specimen to **Register** end**.**
  *Press the **START<=>END** button to reverse the start position and the end position if necessary.*
- Click Stop to complete the preview of the sample.
- Set "**Step Size**" for Z-stack scanning. *This digit suggests the distance in μm between each slide.*



- Click on the LSM Start button to start the recording of the Z Series.
- After the image is acquired, pressing the APPEND NEXT button allows you to perform the image acquisition repetitively under the same condition. Press the SERIES DONE button to compete the image acquisition.

Setting Time Series



- Select **ON** for **Time** in **Series** Tool Window.
- Set the interval by input in how many seconds in **Interval**.
  **If you attempt to set the time shorter than the time displayed in [Scan] (time taken for acquiring 1 cycle image) in [Interval], "FreeRun" appears. In this case, the interval to acquire the image is the time displayed in [Scan].*
- Set the number of image acquisitions in Cycle.
  The time from the start of the T series image acquisition to the end of the T series image acquisition is calculated and displayed in [LSM Total]. Set [Cycle] so that [LSM Total] becomes an appropriate time
- Click on the LSM Start button to start the recording of the Z Series.
- After the image is acquired, pressing the APPEND NEXT button allows you to perform the image acquisition repetitively under the same condition. Press SERIES DONE the button to compete the image acquisition.

Turn off system

Please check if the equipment will be used by other users. Please switch off system if no one books equipment over two sessions (1h) after you.

1. Soak the water objective in MilliQ water for 5min
2. Turn off fluorescent lamp illuminator by pressing "ON/OFF" button for **3 seconds,** the illuminator will show "300" to count down for **5min** for cooling down the lamp.
3. Turn off Chameleon laser by turning the laser key 90° anticlockwise to vertical position
4. Turn off power switch of SIM scanner ④
5. Turn off power switch of Main scanner ③
6. Turn off power supply Unit FV30-PSU ②
7. Exit "FV30S-SW" software and upload your data to server
8. Turn off computer ①
9. When the lamp illuminator **5min** count-down is finish, turn off the power switch of lamp illuminator ⑤ (the switch is at the left back of the box, shown by →)
10. Keep pressing  on touch screen to move the objective to upper limit, then clean up with lens paper
11. Turn off the touch panel controller ⑦ (the switch is at the right back of the box, shown by →)
12. Turn off microscope power ⑥

*Users are reminded to sign on the log sheet before departure.