GE Amersham™ Imager 680

Standard Operating Protocol

I. Imager AI680 User Manual

1. Log in PPMS tracker.
2. Turn on the instrument (①->②)

   ①: Main switch for AI680 (right side)

   ②: Power switch for AI680 (at the front)

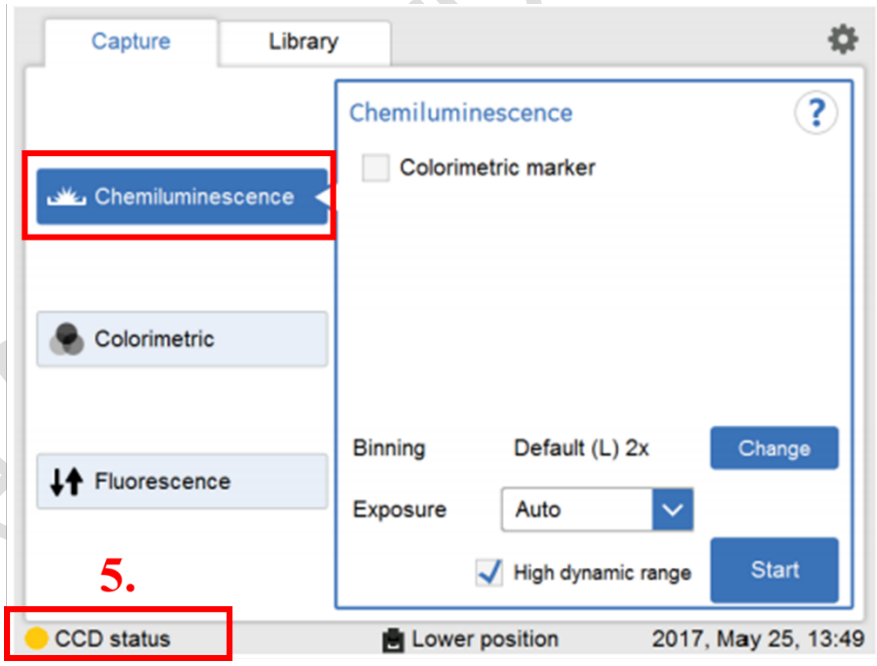
   It takes around 5 mins for the system to be ready.
3. Select the 'AI680' icon in desktop
4. Type in User ID & Password and press 'Continue'.

   User ID: **AI600user**

   Password: **AI600user**
5. Press 'Connect' and the capture screen will be shown.

II. Chemiluminescence Capture

6. Wait for the "***CCD status***" to turn green to begin. Incubate the membrane with ECL while waiting.



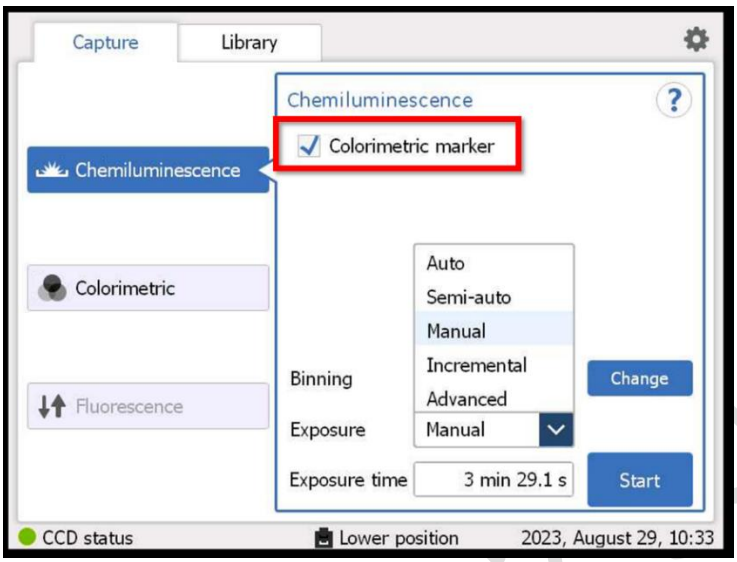
7. Dry the sample and place the sample within the visible marking on the tray. Flip white insert if necessary.
8. Insert the tray in the correct tray position (upper or lower) and close the door firmly.

   Tray position will also be indicated in the lower part of the software.
9. Select the desired exposure mode

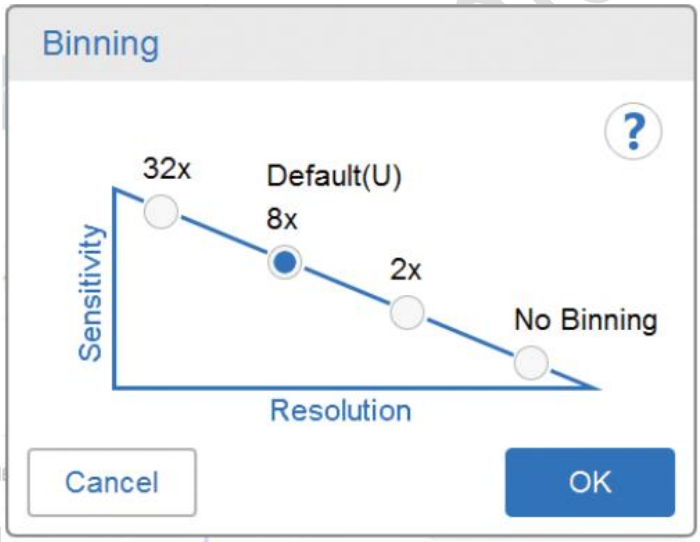
   - Automatic
   - Semi-auto: Pre-Scan to determine desired exposure time
   - Manual: Set own desired exposure time
   - Incremental: Expose >1 images with fixed interval (min. interval:10s)

- Advance (need to uncheck 'Marker'): Fully designated by one, including interval time, exposure time …

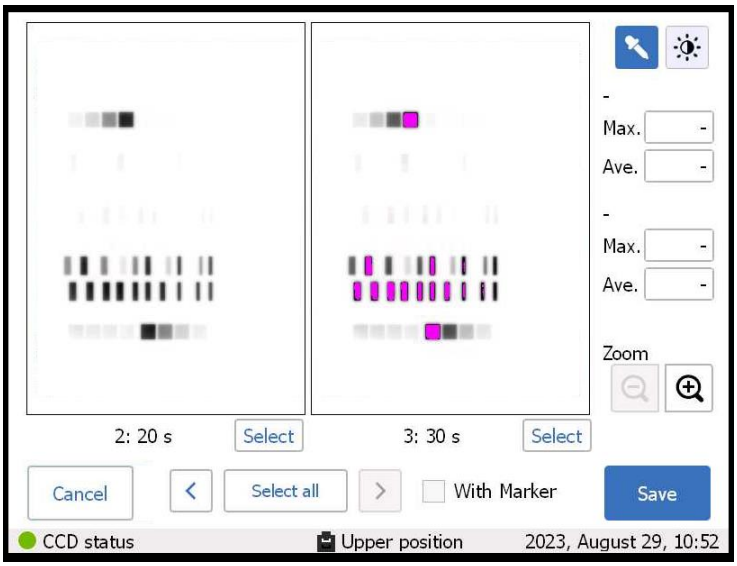
10. Select 'Colorimetric marker' if needed; Choose appropriate binnig; Uncheck 'High dynamic range' for normal capture



- **Low binning**: High resolution & low sensitivity VS **High binning**: Low resolution & high sensitivity.



11. Press 'Start'. A progress bar appears indicating the time remaining of the imaging process.
The image(s) will appear when the exposure is finished. Overexposed signal will be highlighted as purple.



12. Edit image by the right panel. For 'Incremental' exposure, multiple images will be shown. Select which image or images you wish to save.
13. Select the 'Save' button to save the images.

    * You MUST save all the data in "**AI Result**" with file name as

    *'PI initial_user name_date_details'**

    Keyboard is not compatible with the software, so need to input by click on keyboard within the software

    File type: - Capture **without** marker: Gray scale .tif, .jpg

    - Capture **with** marker: Gray scale .tif of sample & marker, .jpg of merged data

    Highlight Saturated pixel: Saturated pixel will be highlighted in pink if selected

***Files will be deleted at the end of every month! Make sure you have exported your data as soon as possible.*

III. Colorimetric Capture

14. Place the sample inside the machine.
15. Choose "***Colorimetric***" mode and press "***Capture***".
16. Save image as step 12.

IV. Image Transformation from 16-bit to 8-bit

17. Open 'ImageJ' in desktop.
18. Open image wanted to transform.
19. Click Image-> Type-> 8-bit.
20. Save as new image. Then the image can be viewed in windows image viewer.

V. Turning off the system

21. Take out the sample tray and remove the sample. Clean the tray with water & Ethanol and dry with kimwipes. Leave the tray outside the machine until the next user use.
22. Close the software. Turn off the power buttons according to the descending order of the numbers (②->①)

    *Wait until there is no running sound after turning off ② before turning off ①.
23. Log out PPMS tracker.

VI. <u>Analysis</u> (optional)

24. To analyze the image, select "***Analyze***".

25. Follow step-by-step to finish analysis

    Lane Creation -> Background Subtraction -> Band Detection -> Molecular Weight Assignment -> Normalization
26. To save the analysis, click "***Save***". The image file will be updated with the analysis information in ***.pdf*** and ***.excel***.
27. To re-open pre-existing images, press "***Library***" and select 1 image **only** and click "***Open***".

VII. ImageQuant

Detail instructions are available in video located in desktop/under 'Help' section of the software.

1. Open the software in desktop.
2. Choose the corresponding analysis mode.
   - 1D gel analysis: Western analysis
   - Analysis Toolbox: For multiple sample types
   - Colony Counting: Petri dish colony counting
   - Array analysis: Multi-well plate assay analysis
3. Open image wanted (must be .tif).
4. Perform corresponding analysis. Can edit image's contrast, merge images, …