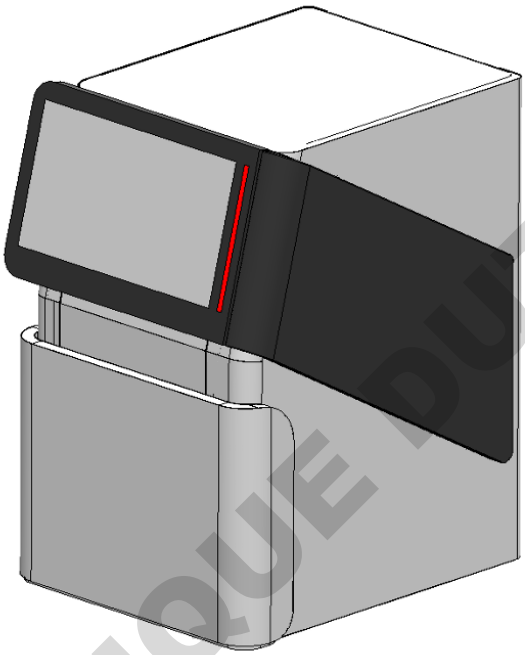
ALLIANCE IRIS QUICK MANUAL

UVITEC CAMBRIDGE

2024/2025



Written by Sacha SAPSFORD, 10/12/2024

UVITEC
CAMBRIDGE

IRIS QUICK GUIDE

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HARDWARE OPERATION

Introduction

The Alliance imaging system is a scientific instrument designed capture chemiluminescence or fluorescence gel or blots images. The Alliance IRIS system uses the IRIS software to control image capture and optimisation for selected applications.

A cooled scientific CCD camera is used to capture high resolution digital images of protein and DNA bands in gels and on membranes obtained by electrophoresis or Western blotting separation methods. The instrument can capture images of chemiluminescent, fluorescent, and colorimetric samples, depending on the system configuration. The instrument can be used for research purposes in the academia and life sciences industry. The instrument cannot be used for diagnostic purpose.

Alliance offers exquisite precision and resolution, which ensures reliable results for both quantification and documentation. The advanced imaging electronics has been developed by our experts especially for your scientific applications. This association of our exclusive electronic, high-quality optics and advanced software delivers outstanding performance. With Alliance, you simply reach the lowest limits of detection on all of your samples.

Warranty

The Alliance imaging system is warranted against faulty construction or defective material **for a period of three years** from the Uvitec Ltd invoicing date. If any defect occurs in the instrument during this warranty period, Uvitec Ltd will repair or replace the defective parts at its discretion without charge. The following defects, however, are specifically excluded:

- Defects caused by improper operation, incorrect use or bad maintenance
- Repair or modification done by anyone other than Uvitec Ltd or the company's authorized agent
- Use of spare parts supplied by anyone other than Uvitec Ltd.
- Damage caused by accident, misuse or disaster
- Corrosion caused by improper solvents or samples

This instrument should not be modified or altered in any way. Modification or alteration of this instrument will:

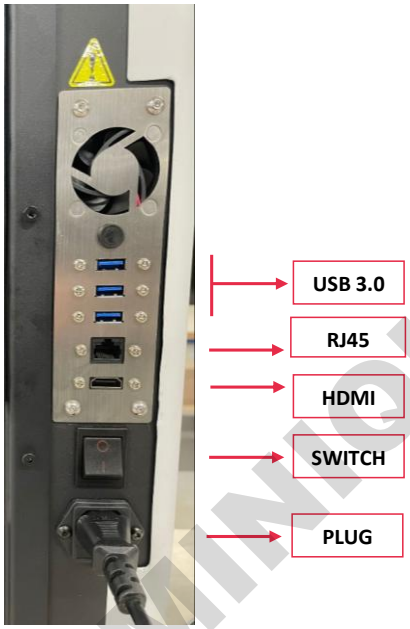
1. Void the manufacturer's warranty.
2. Void the conformity certifications.
3. Create a potential safety hazard.

The light sources (LED panel, UV LEDS …), the filters, the power supplies, the batteries, and the consumables are not covered by our warranty. The use of consumable products or non-original spare parts not recommended by our service department is at the user's own risk and therefore

automatically invalidates the warranty. We reserve the right to decide where the faulty goods will be repaired (in our workshop or elsewhere), and whether or not the faulty part is to be replaced; all other freight charges incurred being at the cost of the purchaser. Returned goods will not be accepted for repair unless previous written authorization is obtained from our service department. A request for authorization must be accompanied by an itemized list of products, model numbers and the corresponding invoice numbers under which they were originally shipped. All returned goods should have a certificate of decontamination. The Buyer must bear all costs and risks incurred during the transportation of the goods from their collection at Uvitec Ltd factory. In the case Uvitec Ltd incorporates some devices or equipment from another supplier in the manufacture of its products, the extent and the duration of the warranty will be those conceded by the suppliers or sellers.

Uvitec Ltd cannot be held responsible for any loss, bodily injury or material accident incurred by any failure of this supply, whatever the origin of this failure may be. Uvitec Ltd is not responsible for any injury or damage caused by use of this instrument for purposes other than those for which it is intended, or by modifications of the instrument not performed by Uvitec Ltd. The responsibility of Manufacturer is strictly limited to its staff and to its own supplies. In the case of dispute, only the commercial court of Cambridge (United Kingdom) shall be competent, even in third party claims proceedings or when there are several co-defendants.

Power Supply



Turning on the system

Alliance IRIS requires only a main standard plug that is to be plugged into its rear end (Figure 1a).

*For a first-time use, please **insert the UV PAD inside the unit BEFORE** turning on the device**

Figure 1. a. Main plug inserted at the rear of Alliance IRIS **b.** Ensure screen appears like above before activating PC **c.** Button to start Alliance IRIS PC

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To turn device on:

1. Flick the button down towards the (I) to switch the darkroom and camera on.
2. Wait till the screen appears as per Figure 1b. before switching on the screen (Figure 1c.).
    a. To indicate PC screen has been activated, the button will be encircled by a blue LED light.
3. The software will launch by itself.
    a. If, however, it does not, a shortcut app located on the computer's desktop can be opened (click twice to open).

Turning off system

To turn device off:

1. Close software
2. Turn off screen
3. Wait till the screen goes blue (Figure 1b)

4. Turn off device by switching off my switch (Figure 1a)

Inserting PADS

Alliance IRIS has introduced a new generation of PADS in the system. Depending on the configuration of the system, it can include the following PADS:

1. UV light emitting diode (LED) pad
2. Chemi pad
3. White LED pad
4. Blue LED pad

The Alliance IRIS contains two different tray levels (Figure 2).

Figure 2. Tray levels. Bottom tray (numbered 1) for UV PAD and Top tray (numbered 2) for Chemi, White and Blue PAD

To recognise the insertion of each pad, a magnetic strip containing a chip can be found on the RIGHT-HAND SIDE. Ensure both the desired pad and the tray position are aligned to guarantee a successful connection between both parts.

UV PAD and UV SCREEN

*PLEASE ENSURE THE SYSTEL HAS BEEN **TURNED OFF** BEFORE COMPLETING THIS STEP*

UV pad is positioned in the bottom tray (tray level 1). To guarantee correct orientation of the pad into the unit, ensure the UV tag, chip and arrow (circled in Figure 3) are facing towards the inside of the unit. To insert the pad inside correctly, slide the tray into the empty compartment and push the pad slowly till you sense the magnetic pull from the tray. Once inside please check the tray is flat and in line. To check, verify the following are aligned (Figure 4)

Figure 3. UV tag, chip and arrow facing the inside of the system. Proceed to slide UV

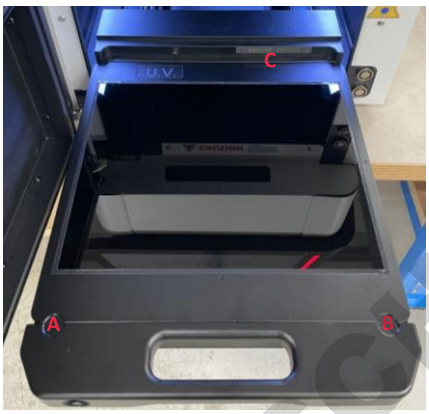

Figure 4. UV table inside unit. Ensure marks (A), (B) and (C) are aligned

To verify successful connection:

1. Turn the system on and software (page 2)
2. Check in 'Light – Filters' tab of the software for the detection of UV 300nm (Figure 5b). Exit tab if pad is detected.
3. Proceed to place a white piece of paper on the UV pad.
4. Place the protection screen provided with the unit onto the UV pad (Figure 5a and 5c). This step will not function without it for safety reasons.
5. Proceed by pressing twice on the UV warning button. The button will be encircled by a red light to indicate activation of UV (Figure 5d).
6. If a blue light appears, a successful connection has been made (Figure 5d). If not, please try again or reach out to Uvitec Ltd for further assistance

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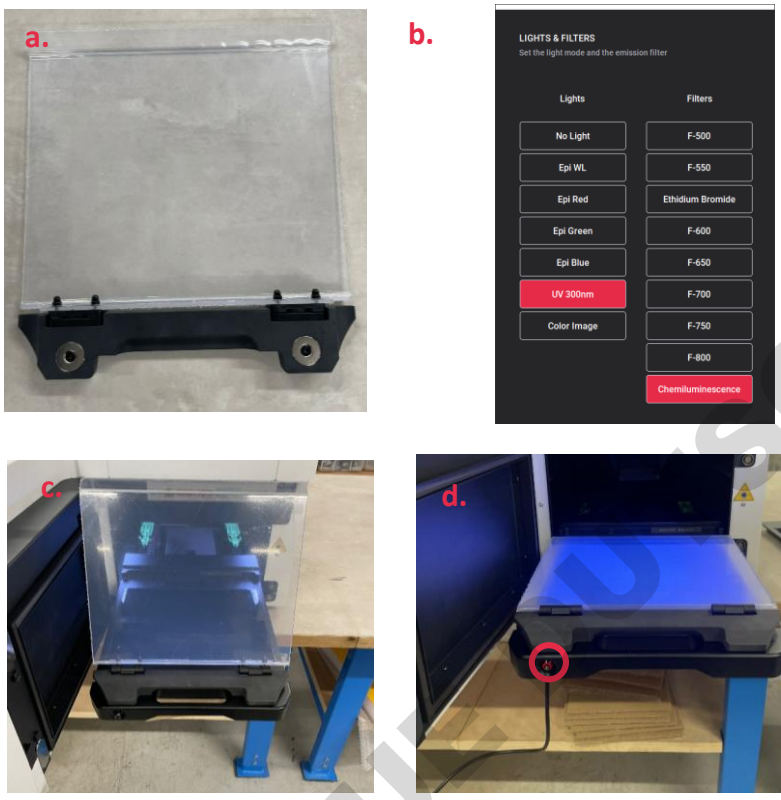

Figure 5. a. Protection screen **b.** UV300nm pad recognised by system **c.** Positioning of protection screen on UV pad d. UV warning button activated (circled) with UV LED light on (blue light)

We recommend not to remove the UV pad from its original position. If so, please switch of the system before remove the pad from the unit.

To maintain the plate in good condition and decrease chances of contamination during use, we recommend to wash the UV plate with either 70% ethanol or water (depending on dye used) on a regular basis.

CHEMI PAD

Unlike the UV PAD, the Chemi pad can be inserted at any time point. This pad is placed at the second tray level (Figure 2. And Figure 6a.) – above the UV pad. Similarly, to the UV pad, to ensure correct positioning of pad, ensure the label 'Chemi' and its chip are facing towards the inside of the unit. To verify successful insertion of pad:

1. Go to IRIS software

2. Select ‘Light and Filters’
3. Check ‘Chemi Pad’ appears in the Light column (Figure 6b.)

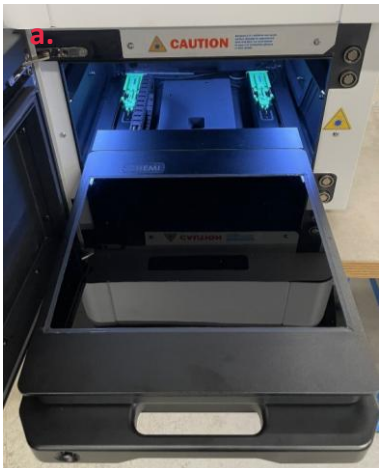

Figure 6. a. Positioning of chemi pad in the system **b.** Chemi Pad recognised by system and software

For applications, this table will cover both chemiluminescent and epi-fluorescent applications.

To remove the pad, place your left and right hand slightly below the chemi pad and lift up before sliding the pad towards you (Figure 7.).

Figure 7. Removal of Chemi pad

To maintain the plate in good condition and decrease chances of contamination during use, we recommend to wash the UV plate with either 70% ethanol or water (depending on dye used) on a regular basis.

WHITE/BLUE PAD

Similarly, to the chemi pad, the white/blue pad is positioned at the second tray level (Figure 2. And Figure 8a.) – above the UV pad. Like the other pads, to ensure correct positioning of pad, ensure the label 'White' or 'Blue' and its chip are facing towards the inside of the unit. To verify successful insertion of pad:

1. Go to IRIS software
2. Select 'Light and Filters'
3. Check 'White Pad' or 'Blue Pad' appears in the Light column (Figure 8b.)

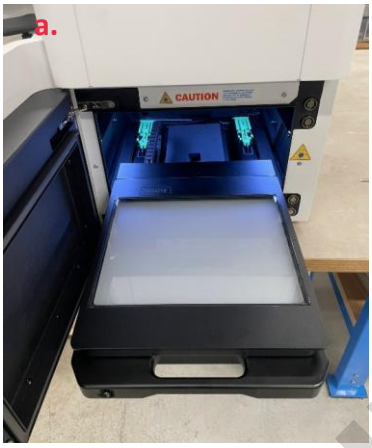

Figure 8. a. Positioning of white/blue pad in the system **b.** White/Blue Pad recognised by system and software

For applications, white pad will cover basic Coomassie staining applications whereas the blue pad will cover DNA gels that are excited in the 400-500nm wavelengths (SafeDyes for example).

To remove pad, please follow the guidelines provided in the chemi pad section.

To maintain the plate in good condition and decrease chances of contamination during use, we recommend to wash the UV plate with either 70% ethanol or water (depending on dye used) on a regular basis.

ChromaScan

Alliance IRIS make use a novel technology known as ChromaScan. Details on the mechanism and positioning of the ChromaScan will be eventually released with the full Alliance IRIS manual. In this QUICK GUIDE, a simple introduction to the insertion of the ChromaScan excitation light source and emission filter are presented.

EXCITATION LIGHT SOURCE

Figure 9a. presents the excitation light source of the Chromascan for epi-fluorescent applications. Up to 8 of these individual light sources can be inserted on the mechanical wheel located within the front part of the dark room of IRIS (Figure 9b.)

Figure 9. a. ChromaScan Excitation Source. Red arrow indicates location of ChromaScan wheel **b.** Final position of ChromaScan Excitation source on the mechanical wheel

In order for it to be positioned as per Figure 6b, there are a few important steps to follow (Figure 10):

1. Ensure system is turned on and software is open
2. Access settings > spectra (Figure 11.)
3. Grab your first ChromaScan light source by using your left hand and placing fingers in the provided finger holdings (Figure 10a)
   - Ensure the LED part is facing down towards the plate and not towards the mechanical device
4. With the help of your right hand, insert the chip by sliding it in carefully into a slot located on the right side of the system (Figure 10b-d)
5. Proceed by pushing in the chip till you do not see a gap (as per Figure 10d)
6. With your left hand, push the left end of the light source into the corresponding lane till you hear a click. To check if correctly positioned, slightly rotate the wheel up and down (Figure 10e and 10f) to ensure it is tightly in position

7. To place other light sources, manually rotate the mechanical wheel to spaces that are empty and repeat steps 4-6.
8. When complete, press 'rescan' on the software and check all the capsules have been recognised (Figure 11.)

Figure 10. Insertion of excitation light source on mechanical wheel a. Orientation of ChromaScan excitation source **b.** Insertion of right side of the light source into the right-hand side (RHS) of the mechanical wheel **c.** and **d.** close up of the chip being inserted into the RHS of the mechanical wheel **e.** and **f.** close up of the left-hand side (LHS) of the light source being inserted into the LHS of the mechanical wheel

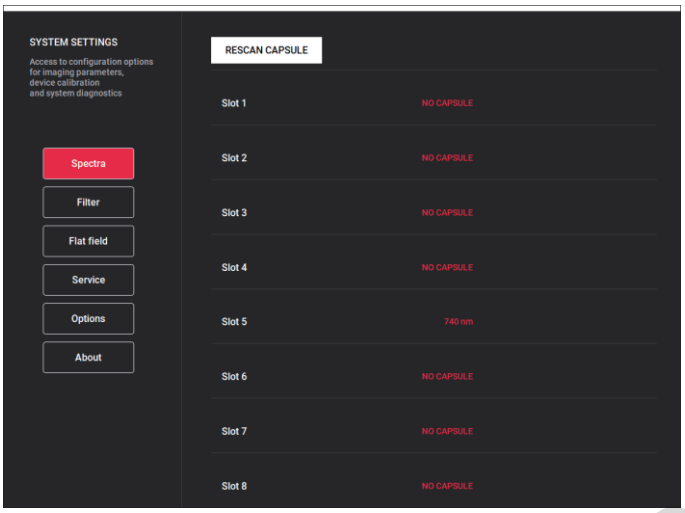

Figure 11. ChromaScan option in IRIS software settings

To remove the excitation light sources, conduct steps 3-6 in reverse. At all times, ensure that a rescan is done to inform software and the unit that there has been a physical change made.

Do not travel with the excitation sources inserted inside the unit

EMISSION FILTER

Alliance IRIS has integrated emission filters to its unit that are smaller and magnetic, facilitating the insertion or removal of these narrow bandwidth filters. If the need to add filters is required, please follow the following guidelines:

1. Turn on system and software
2. Access software settings and select filter (Figure 12a)
3. Identify an empty position and select 'replace filter' (Figure 12a)
   - Please wait for camera to lower before opening door
4. Once camera has lowered, open the door. You will now have direct contact with the camera.
5. On the RHS is located a small door. Opening the door will provide you access to the filter wheel. Open this first (Figure 12b).
6. Using your right hand, grab your filter, avoiding any and all contact to the lens, and carefully insert the filter in the gap presented in the filter wheel. Ensure that the filter is aligned with the wheel. To check, run you finger around the corners of the filter to verify the flatness (Figure 12c-e). In the circumstance the filter is dirtied, use a clean and unused cloth to wipe fingerprints.
7. Return to the software and select the inserted wavelength used (Figure 12f).
8. Close the door of the filter wheel located on the camera
9. Repeat steps 3-8 for all filters that are to be added. Click out to return to main page of software.
   - Please wait for camera to return to its original position before using the system

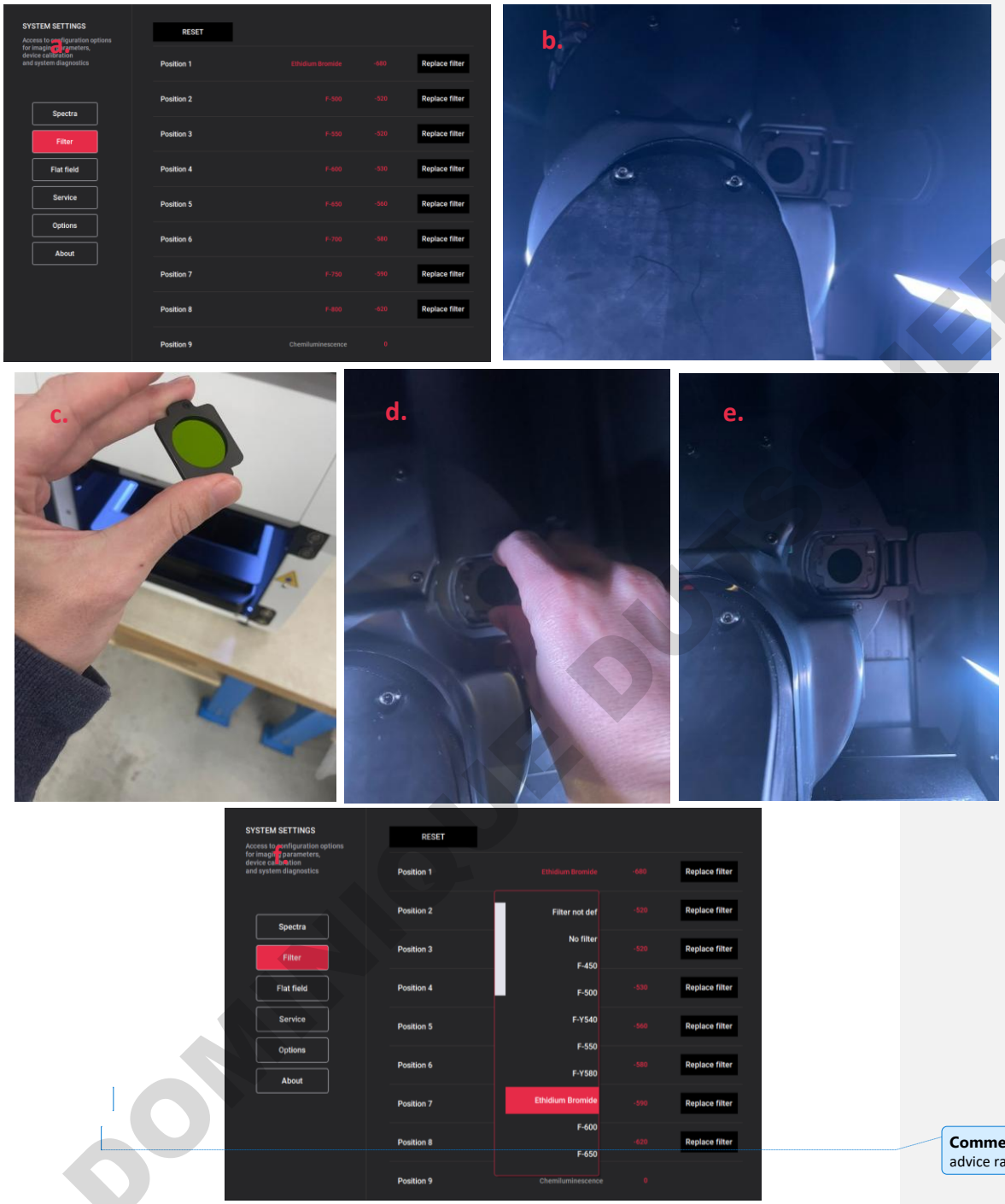

Figure 12. Insertion of emission filters. a. Settings access **b.** Location of filter wheel door on right hand side of camera **c.** standard emission filter **d.** insertion of emission filter using right hand **e.** final result **f.** selection of filter on software

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To remove a filter, please follow the following:

1. Turn on system and software
2. Access software settings and select filter (Figure 12a)
3. Identify the filter you desire to remove and select ‘replace filter’ (Figure 12a)
4. Please wait for camera to lower before opening door
5. Once camera has lowered, open the door. You will now have direct contact with the camera.
6. On the RHS is located a small door. Opening the door will provide you access to your desired filter.
7. Using your right hand, proceed to place your hand directly behind the RHS of the camera to identify a circular push button. Once identified, press the button using your right hand to extract the filter out of its current position. Using your left hand, proceed to take the filter away from the filter wheel. Be careful to not touch the filter lens (Figure 13.)
8. Once removed, close the door of the filter wheel on the camera
9. Return to the software and change the filter to ‘no filter’ unless otherwise stated (Figure 12f.)

Figure 13. Removal of emission filter. Using your right hand, identify the push button located directly behind the filter wheel.

SOFTWARE OPERATION

Introduction

The Alliance IRIS software is a licence-free software for image acquisition and analysis. The current version of the IRIS Software contains only the image acquisition capacity (2024). Hence, in this quick manual only this section will be explained.

Turning on and off software

Upon activation of PC (Figure 1c), software will launch automatically. If not, a desktop short cut has been created, simply double click to open.

Once launched, the software will automatically load the following:

1. USB components to ensure camera and dark room detection
2. Camera cooling to activate cooling of the camera – this is a 2-step process. Cooling takes approximately 3-minutes. During this time, you will not have access to the software.

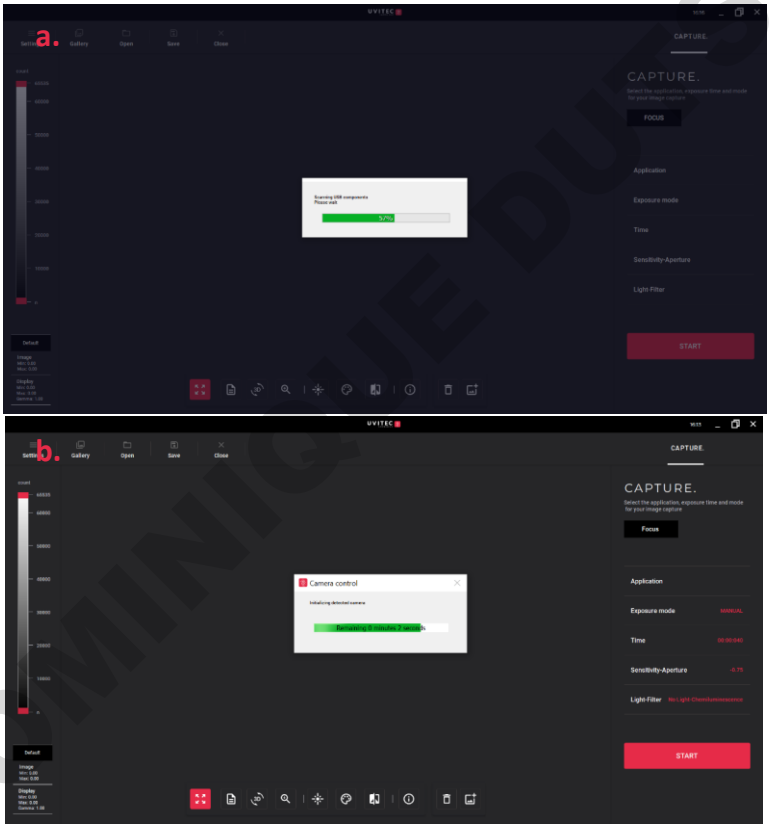


Figure 14. Activation of camera, dark room and cooling. a. USB components to connect camera and dark room are activated **b.** A 2-step process to activate cooling is activated

System settings

To access the configuration of the system, the systems parameters, the device calibration and system diagnostics, the systems settings is located on the top LHS of the settings.

SPECTRA

To view or modify the light excitation source, please access this tab. All information on how to add or remove a light source is provided in the Hardware operations – Figure 10.

FILTER

To view or modify the emission filter, please access this tab. All information on how to add or remove an emission filter is provided in the Hardware operations – Figure 12. There is no need to modify the focus values as these have been automatically set. To reset these values to its origin number, proceed by pressing 'reset'.

FLAT FIELD

An additional feature brought by the Alliance IRIS is the possibility for the researcher to ensure homogeneity in their images by running a flat fielding check. This process is only important if the researcher woks with epifluorescence. For this check-up, please contact your local distributor to assist.

Uvitec Ltd will provide to all distributors two plates (pink and black) that will be of importance for this step. Please follow the guidelines below:

1. Place the black tray on the Chemi pad for wavelengths 460nm, 520nm, 640nm. For wavelengths 680nm and 780nm place the pink tray instead
2. On the software, access settings > flat field
3. Select the desired wavelength
4. Select the desired emission filter
5. Press Start
6. Once the process is over, flat fielding enhancement has been completed

SERVICE

The Alliance IRIS has its own system diagnostics. In the unlikely event the user identifies a bug or hardware malfunction, select 'Park system' in the service section of the settings. The system will lower its camera and proceed to diagnose the matter at hand. Once diagnosis is complete, a tab will appear. Save this tab and send it to your local distributor or the manufacturer at support@uvitec.co.uk

OPTIONS

X

ABOUT

To access the configuration of the device, please access this tab.

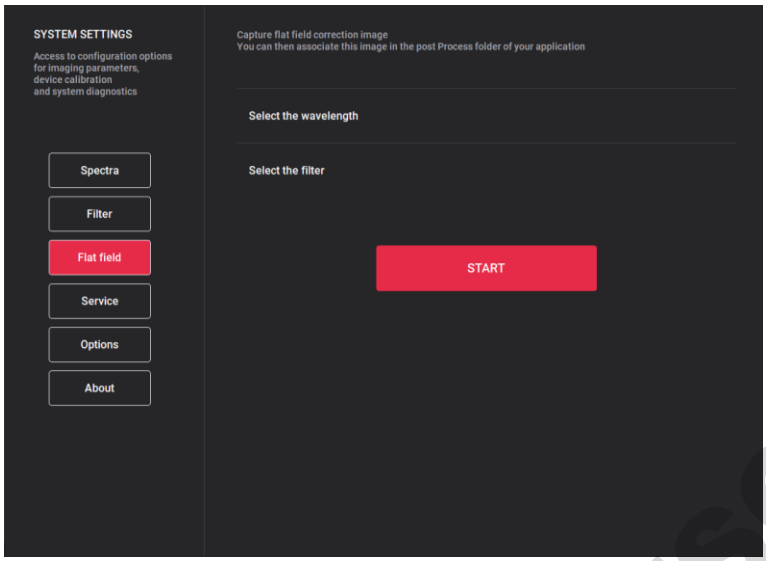


Figure 15. Systems settings. Select tab desired, in this case, flat field tab was selected.

Focus

In order to view, correct and position correctly your sample, the function focus is to be used. This feature allows you correct the following:

1. **SAMPLE SIZE**

This feature helps determine the field of view (FOV) desired based on the width and sample size. There are 8 different possible FOVs to select from: 7x6cm, 10x8cm, 12x10cm, 15x12cm, 17x14cm, 20x16cm, 22x18cm, 24x20cm (Figure 16.)

To change the FOV, select the desired size sample. Allow time for camera to make re-adjustments before selecting another choice.

In epi-fluorescence, the range will be limited between 17x14cm to 24x20cm to ensure homogeneity in sample.

In chemiluminescence we recommend to be as close to sample to minimise the loss of photons during acquisition.

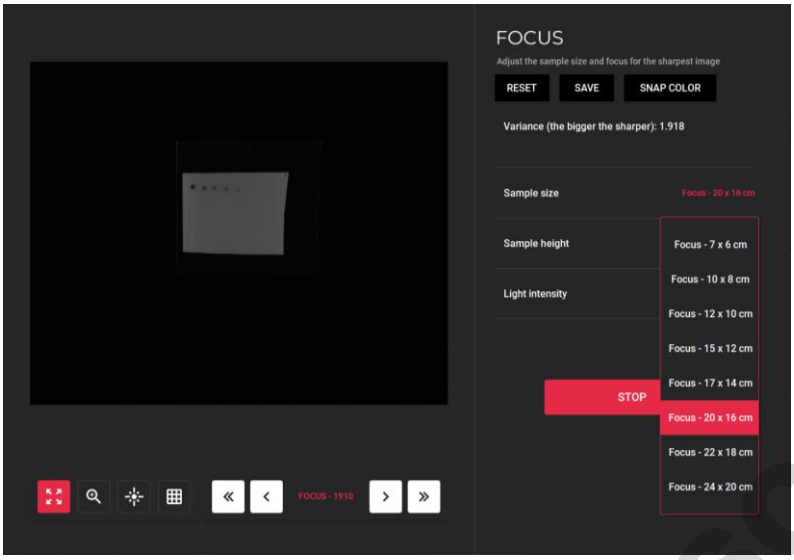


Figure 16. Focus – sample size. To select desired FOV, select sample size and choose desired camera distance. Focus will be done automatically on the selected distance.

2. SAMPLE HEIGHT

To modify the focus as a result of a change in the sample height (i.e., 96-well plate), changes can be made here. Alliance IRIS software's allows thus far the maximum height of 4cm (Figure 17.)

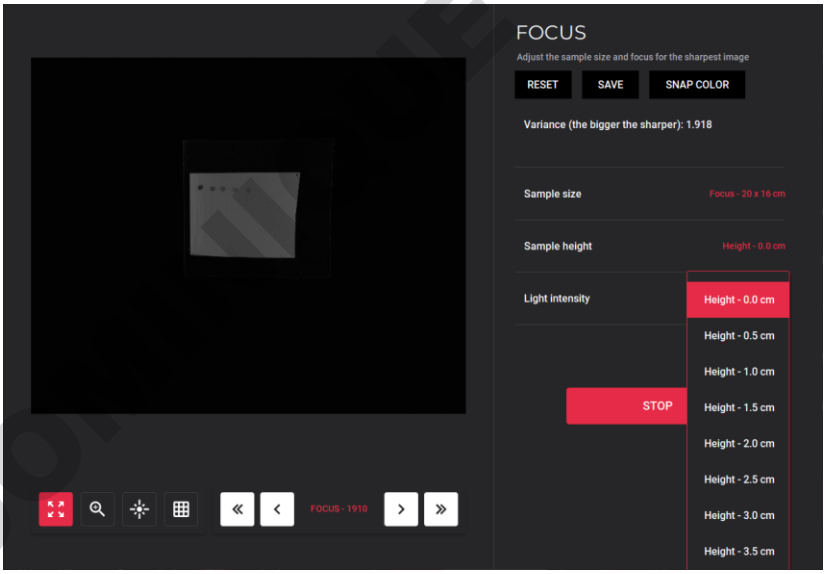


Figure 17. Focus – sample height. To select desired height of sample, select this choice and the height (in cm) of your sample. Focus will automatically adjust to this change

3. LIGHT INTENSITY

To correct the intensity of the white light, intensity of light can be changed here. Intensity can be changed to: 10%, 25%, 50%, 75% and 100%

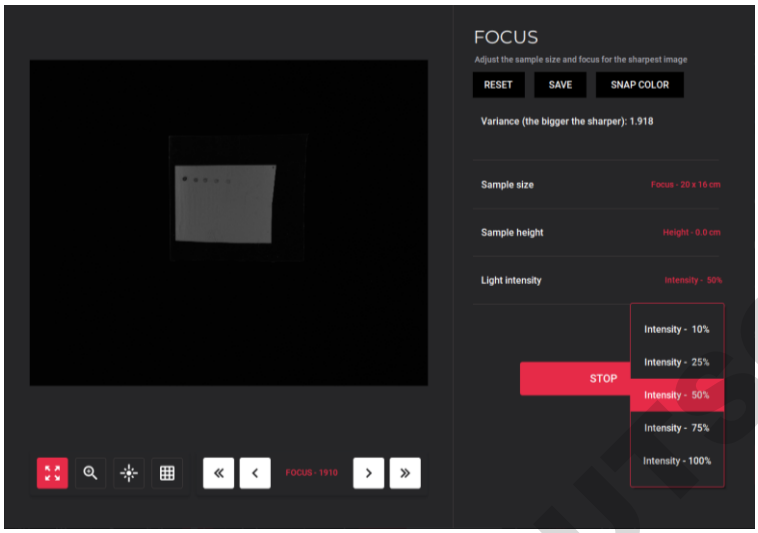


Figure 18. Focus – light intensity. To select light intensity, select between the 5 options; 10%, 25%, 50%, 75% and 100%

4. FOCUS

To manually modify focus, the following buttons are provided (Figure 19.):

- << >> to change focus value by a total of 10 focal points
- < > to change focus value by a total of 1 focal point

It is important that if you decide to make these changes, to allow the camera time to make these changes before increasing or decreasing the focal point.

5. OTHER

The focus settings include other features to assist and facilitate your acquisition (Figure 19.):

- Zoom: enhance image view
- Saturation: signal intensity has reached full intensity (displayed in pink)
- Grid: linearise sample
- Reset: to reset focus to its original value
- Save: Save settings in protocol (application)
- Snap Colour: image sample in Red, Green and Blue (RGB)

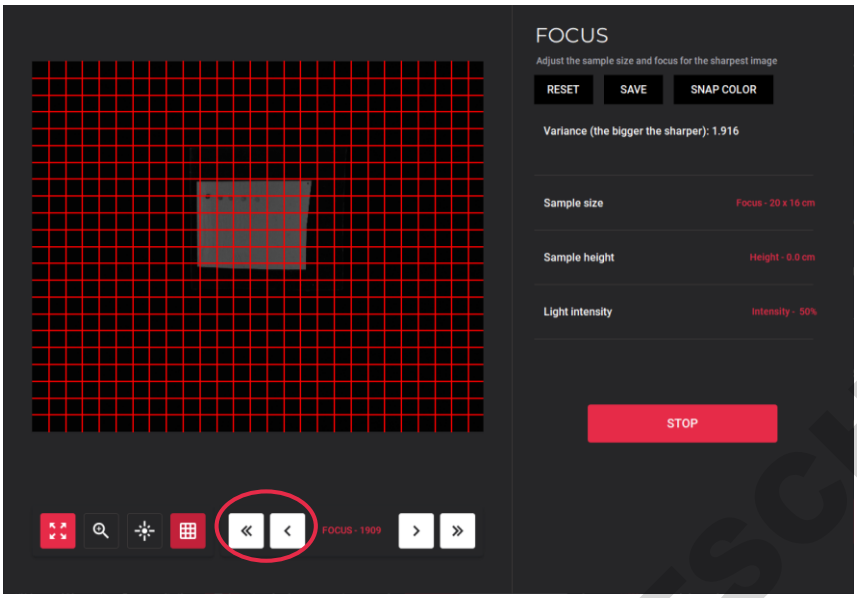


Figure 19. Focus – general features. To exemplify, the grid option has been selected. Circled in red are the options to manually change the focus by either 1 focal point or 10 focal points. Options to change zoom, saturation are located on the bottom left whilst to save, reset or take a photo in colour, buttons are located on the top right-hand side.

Capture your image

In order to take the best photo and prepare your photo for publication or analysis, there are few key steps that need to be done.

Choose Application Type

Depending on your application, the correct option needs to be selected.

Please be sure to select the correct tray for the application that is to be used

1. To select protocol, click on Application
2. Alliance IRIS provides two columns; Standard (pre-defined protocols) and Custom (personalised protocols)
3. When working with the following, please select:
    - Chemiluminescence: select protocol termed 'luminescence'
    - UV: Select protocol termed 'UV gel'
    - Coomassie gels: Select White light gel
    - Epi-fluorescence: Select protocol corresponding to desired wavelength
    - Marker: Select 'white light EPI'

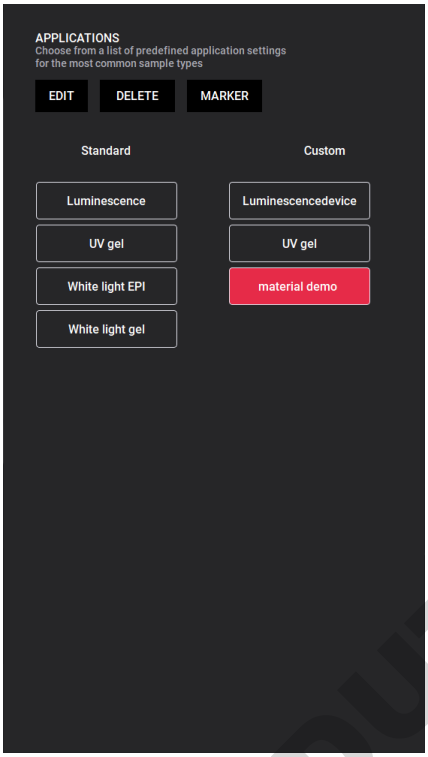


Figure 20. Application. Access to application provides you towards pre-defined and custom-made protocols

Edit Application Type

With Alliance IRIS, the capacity to create or edit protocols based on a pre-defined protocol can be done. To do so:

1. Select Application
2. Click on Edit

Once on the editing page, as a user, you have access to the following options: imaging mode, auto exposure, manual exposure, serial exposure, post process, display, general and focus.

- Imaging Mode: this tab provides you access to how the protocol will be set and how the image will be taken i.e., automatically set up the field of view and desired sensitivity
- Auto exposure: determine the how to operate automatic acquisition
- Manual exposure: determine how to operate manual acquisition
- Serial exposure: determine how to operate serial exposure
- Post Process: Image correction i.e., overlay to include marker
- Display: Presentation of image i.e., inverse of image

- General: overview on device specifications
- Focus: Focus values used in protocol

Following changes, press 'save' and name protocol to your desired taste. Once saved, the protocol will automatically appear under the custom column.

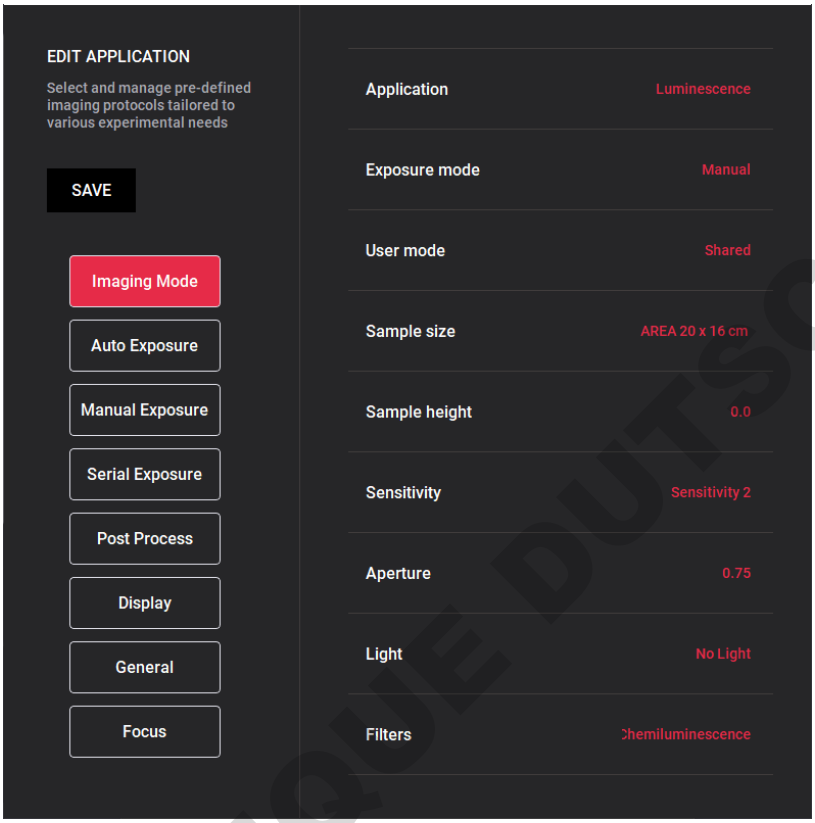
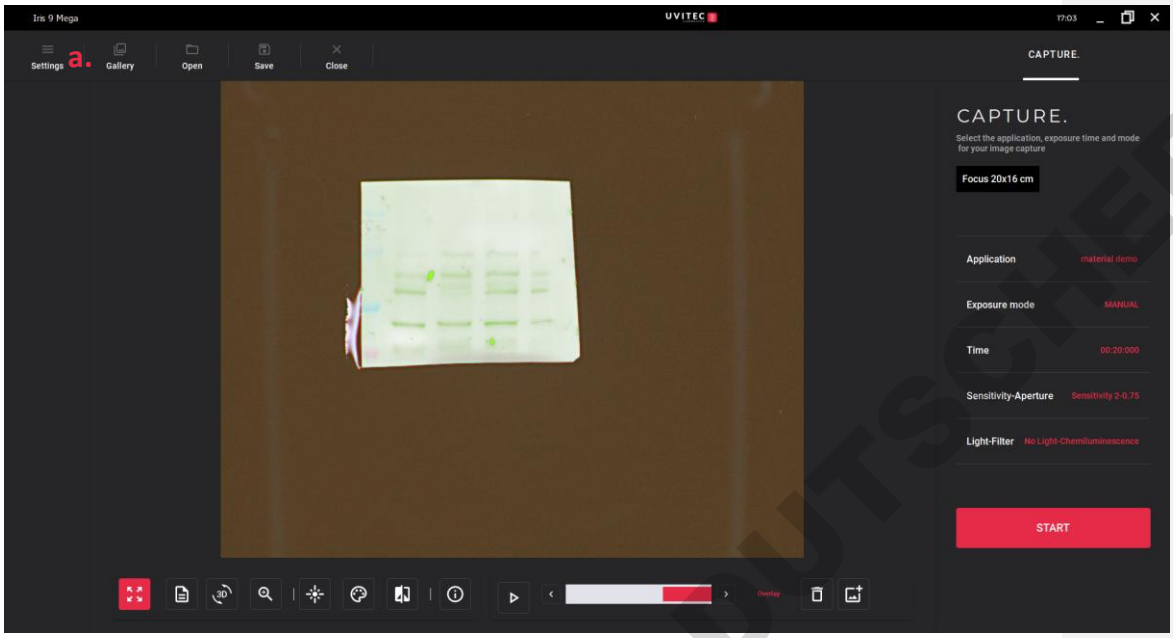


Figure 21. Editing Application. Tab 'edit application' provides access to intricacies of image, allowing users to adapt the methodology of the photo to their application as well adding additional features including the inclusion of the marker or coloured images.

- Marker addition: to add an overlay that includes your marker follow these steps:

1. Edit Application
2. Select Post Process
3. Select Marker – choose between black and white (BW) or coloured marker
4. Save protocol
5. In the main application tab, the words 'marker' will be highlighted in white (Figure 22b.)
6. Choose exposure mode and start

7. The Alliance IRIS will provide you 3 photos: signal image, marker image and overlay image
8. Save images



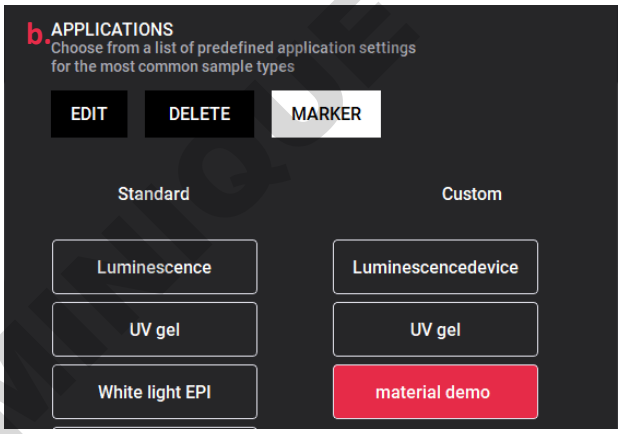


Figure 22. Marker Addition. a. Final overlay option – all 3 photos (signal, marker and overlay) can be accessed by clicking the following < > circled. **b.** To ensure marker has been successfully added, it will appear highlighted in white in the application tab

Acquisition Mode

Before taking an image, the acquisition mode, found on the main page of the software allows you to capture the image in 3 different ways:

1. *Automatic*: The Alliance system automatically calculates the optimum exposure time.
2. *Manual*: The Alliance system allows the user to select the desired exposure time
    - *Time definition mode:* User defines a desired time ranging from 1 minute/second (min/sec) to 60-mins
    - *Time Scroll Mode*: User gets a live-preview of sample over a duration maximum of 10 secs (recommended for UV gels or White light gel applications)
3. *Serial*: The Alliance system captures a series of images within a range of exposure times configured by the user.
    - *Incremental:* The user can define the exposure time of the first image and the last image. The system will calculate the time to be added to each photo between the range provided – this will depend on the number of images that are to be taken.
    - *Repetitive:* The user defines the number of images to be taken at a certain time X of exposition. Each subsequent photo will be taken for the duration of the time X provided. Signal can also can accumulated on top of each other to amplify desired signal (option)

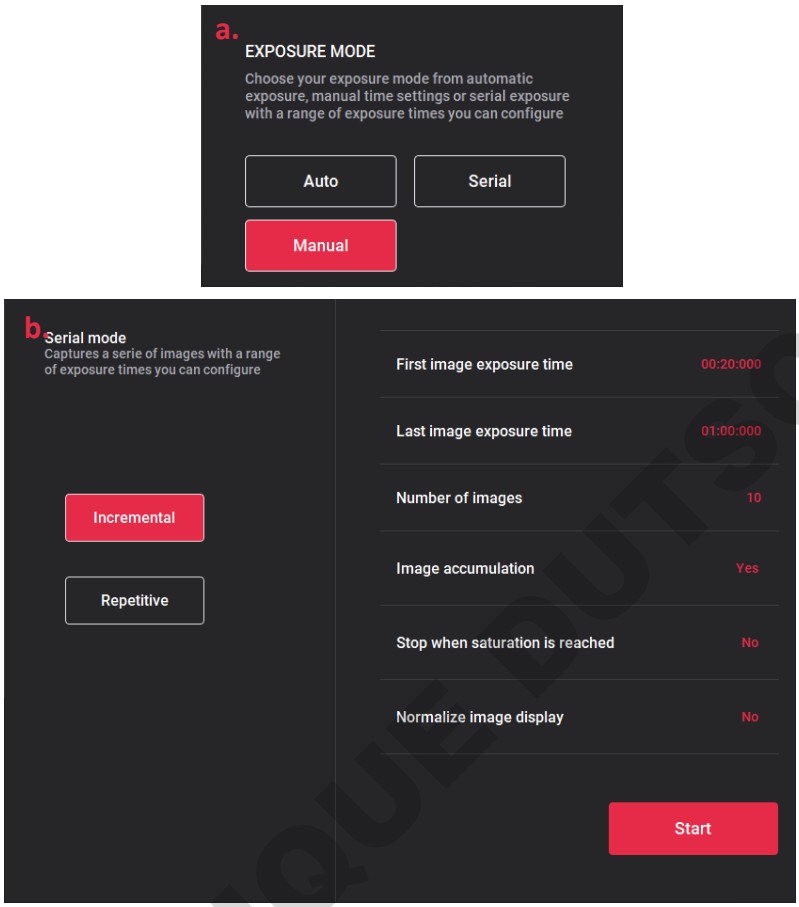


Figure 23. Exposure mode(s). a. Select between automatic, manual and serial. **b**. In serial, there are two options; incremental and repetitive. Incremental allows users to define an initial and final exposure time where the software will automatically calculate an increment time between defined times. Repetitive will take a number of photos at the same X exposition time provided

Sensitivity and Aperture

Whilst in most cases the sensitivity and aperture are automatically pre-defined in the protocol, users still have access to these features.

By selecting 'sensitivity & aperture', you can adjust the camera's aperture and binning settings to increase or decrease the camera's sensitivity as needed for your defined application.

- Sensitivity 1: High Sensitivity (native binning)
- Sensitivity 2: Super Sensitivity
- Sensitivity 4: Binning 2x2
- Sensitivity 8: Binning 4x4
- Preview mode: Epi-white light

Commenté [SS5]: Tristan: Definition

For aperture, the lower the value the greater the amount of light is being welcomed in by the camera. Hence, a value of f/0.75 represents the largest aperture, while 16 represents the smallest. If saturation or signal is strong, we recommend closing the aperture (i.e., from f/0.75 to f/2.8) to decrease the amount of light entering the camera.

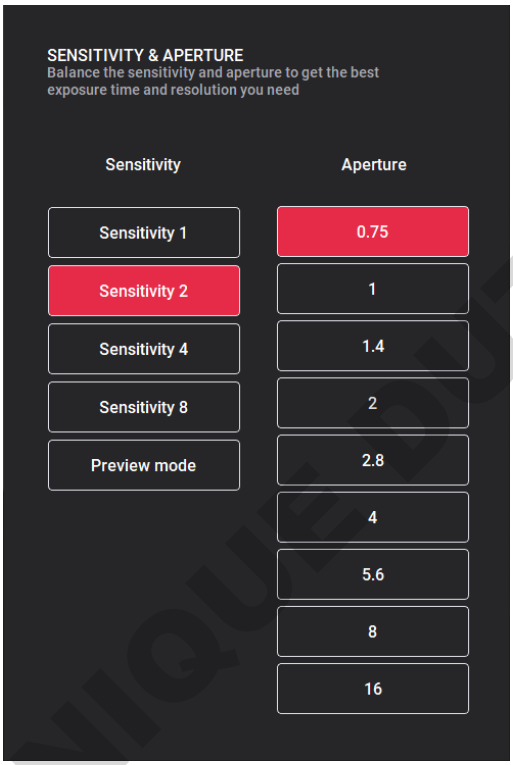


Figure 24. Sensitivity and Aperture.

Light and Filter

In the case you necessitate a change of filter or excitation light, you can do so by accessing the 'Light & Filter' tab. Click on 'Light & Filter'. Once on the tab, select the desired excitation light and emission light corresponding to your dye. Below is a short list of the recommended combinations commonly seen:

Application	Light	Filter

Chemiluminescence	No Light	Chemiluminescence
UV gels	UV 300nm	Ethidium Bromide (F590)
Blue wavelength	C480nm	F500/F550
Green wavelength	C520nm	F600
Red wavelength	C640nm	F700
NIR wavelength	C690nm	F750
IR wavelength	C780nm	F850

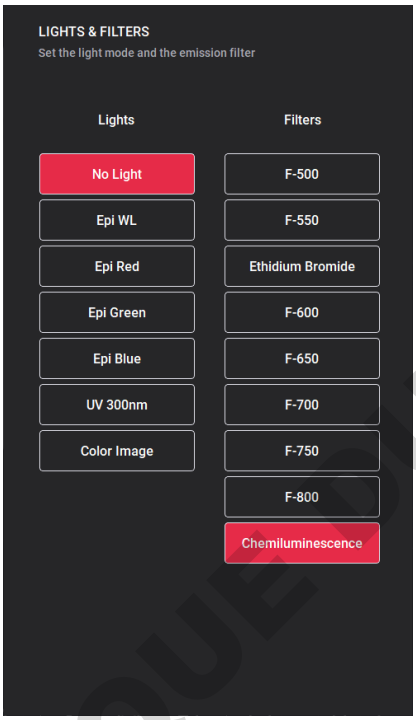


Figure 25. Light & Filters.

Area of interest

The feature, area of interest, allows the user to define an area on the test image to calculate the auto-exposure time required for the specific part selected.

To access this option, exposure mode needs to be in automatic.

Once you press Start (in automatic mode), the system will propose a template with the optimum exposure time. The feature, area of interest, will appear on the top right-hand corner.

To manipulate the area of interest desired, select the white squares on the outside of the box to align and define your area of interest. When contempt with the choice, press continue to acquire final image.

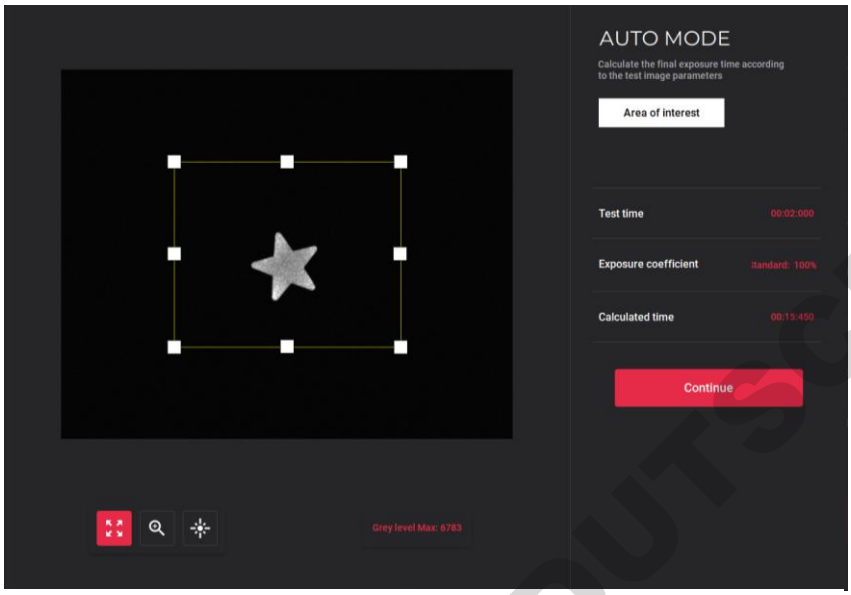


Figure 26. Area of interest. When in automatic mode, the option to select an area of interest will appear on the top right-hand corner. To define parameters, select the small white boxes to re-adjust the zone of interest. The calculated time will change according to its new area of interest.

Acquire the image

In order to acquire a photo:

1. Select Application
2. Depending on exposure mode, select one of the following: Auto, Manual, Serial
3. Click 'Start'
4. In automatic, a template with the optimum exposure time will appear, press 'Continue' to achieve final image
5. In serial, a template to indicate the desired needs (Figure 22) will appear. Save files to desired folder and press 'continue' to achieve final image(s)
6. In manual (time scroll mode – *Figure X*), play with time of exposition before pressing 'stop' to achieve final image

Image contrast

Grey scale represents a specific intensity of light. In the Alliance range, the maximum number of grey levels is 65, 635 levels of grey (or for any 16-bit camera). Factors, such as aperture,

camera cooling and resolution can impact the final value – impacting 1) contrast or brightness of the image 2) the capacity to quantify correctly. To access the amount of grey scale captured, a bar on the left-hand side along with the image percentage (%) and display histogram are provided (Figure 27.). The bar (1) provides the user the ability to adjust the grayscale to help render invisible bands visible or to help remove additional background noise, seeing that the naked eye can only perceive 254 shades of grey. The image % (3) provided calculates the number of grey scales captured out of 65,635 levels of grey as a percentage whereas the display option (4) provides an idea of the full range of pixels captured by the camera. As a rule of thumb, an image with > 80% grey scale exemplifies a good quality photo as it will assure sufficient information for the user to 1/ play with the contrast of the image but 2/down-stream quantification. Info (2), presents the intensity of a single pixel depending where the mouse cursor is placed on the blot.

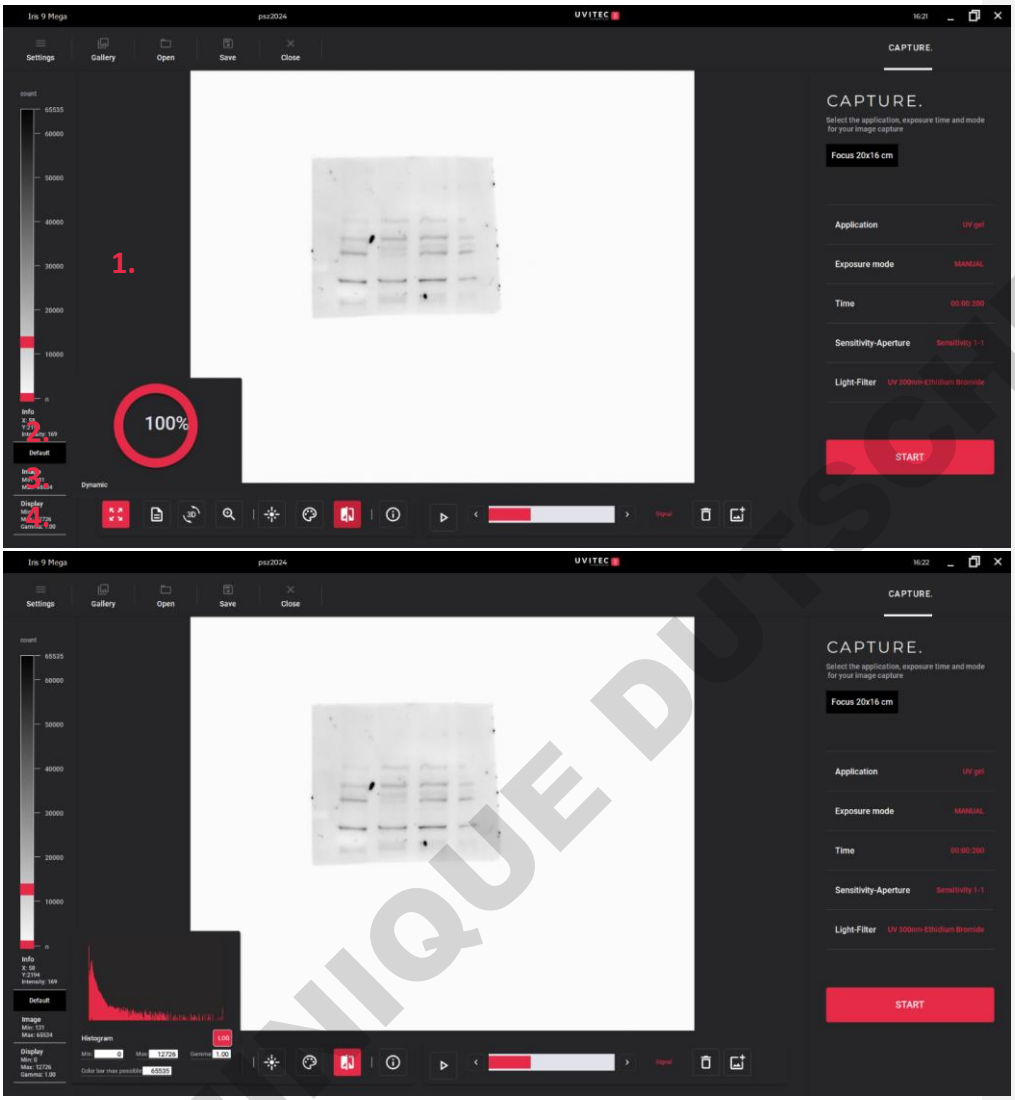


Figure 27. Grey Scale Display. On the left hand-side the grey scale bar (1) can be readjusted by moving the two red squares along the bar. By selecting the image display percentage (%) (3), a representation of the % of shades of grey captured appears. To access the histogram and visualise the intensity of all grey levels selected, display (4) feature is provided whilst info (2) indicates the intensity of light of a single pixel at a certain region of interest marked out by the marker

Save and print

To save the photo, select the option 'save' located on the top left-hand side of the software (Figure 28.). When saving there are different formats to select from:

- *16-bit tagged image file format (*.tif)* – recommended for analysis
- *Open platform 16-bit tagged image file format (*.tif)* – recommended if image has been edited. The file format is a multi-layer based format containing the image as displayed in the software (i.e. edited image) in 8-bit format, and the raw data image in 8-bit format containing image settings and the GLP data
- *Bitmap File (*.bmp)*
- *Jpeg (*.jpeg)* - compressed

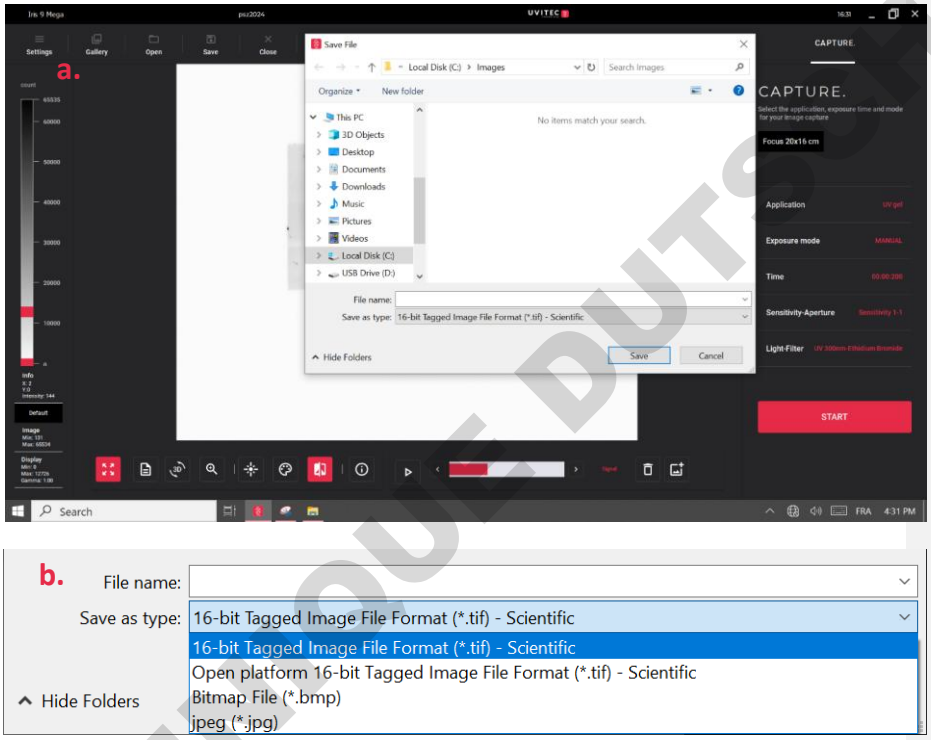


Figure 28. Saving images. a. Save option located top left-hand corner of the software. b. Different formats to save documents

Visualise your image:

Gallery

The Gallery function, located on the top left-hand corner, saves automatically the first 250 photos taken (whether it has been saved or not). Once it reaches 250, it will start deleting the first initial photo taken on the device.

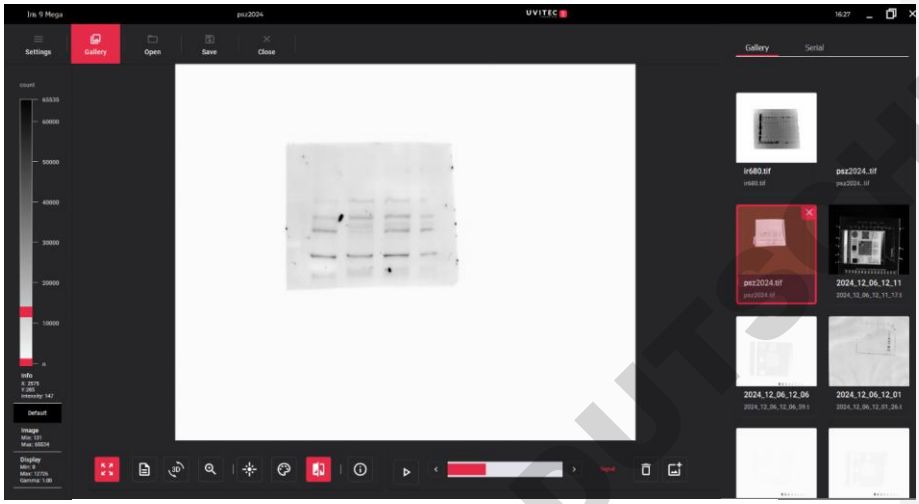


Figure 29. Gallery view. Access old and recent photos via Gallery function

Colour image

For users who would like to colour their images, there are two different ways to do so:

1. Automatic via protocol
    a. Select application
    b. Edit protocol
    c. Display
    d. Select colour desired in the 'display image'
2. Manually via main page of the software
    a. Select the paint motif and select colour desired

Recommend: to save photo in Open Platform to save changes made to images if coloured

Colour options: Red, Green, Blue, Multicolour (intensity), black/white

Figure 30. Coloured image. Via paint motif, blot can be coloured based on personal preference

GLP

To access information regarding the image, select the symbol (i) to view application and image protocol. All these information is automatically saved in the image and final report in analysis.

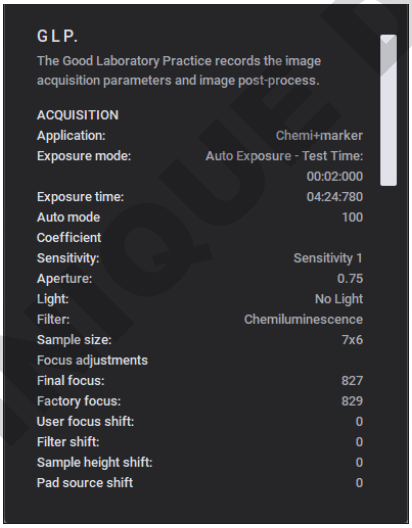


Figure 31. GLP.

Inverse and Saturation

In order to change the background of the image from black to white or white to black, the inverse function can be used. View figure 32 (1).

To indicate saturation (pixel intensity > 65, 635 grey levels), select the button in figure 32 (2). Saturation is indicated in pink.

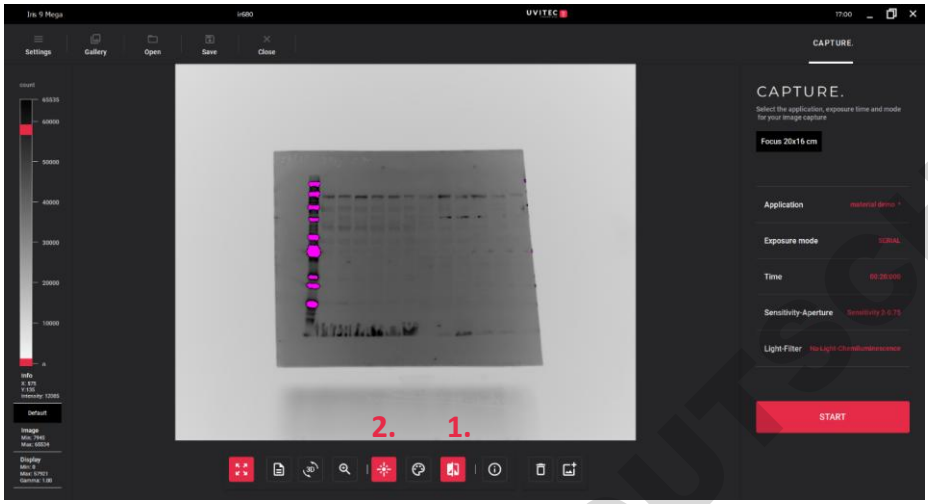


Figure 32. Inverse and Saturation. (1) to inverse image and (2) to indicate saturation viewed in pink

Notes

To add any additional comments to the photo, select the notepad. Any comments made will be saved in the final report of the image (Figure 33a.)

3D view

To view sample in 3D, select the function 3D. Values are based on grey scale captured (Figure 33b.)

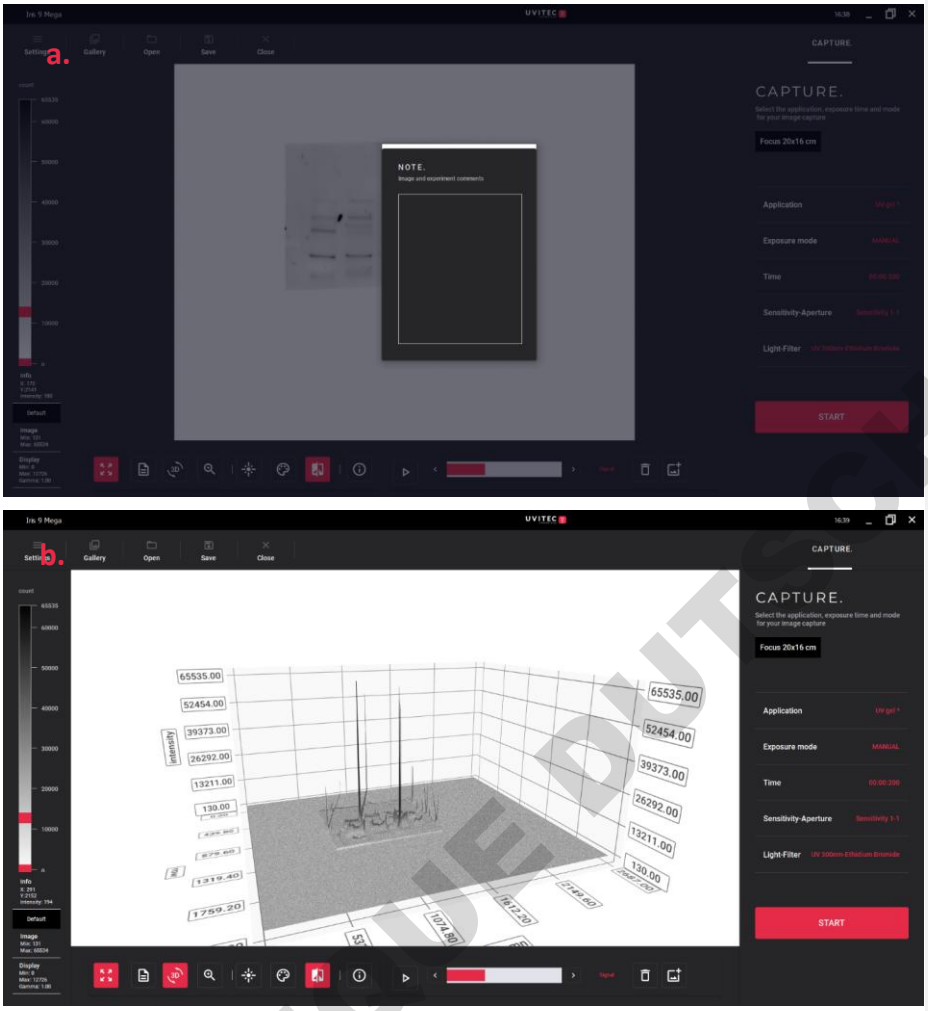


Figure 33. Notes and 3D view. a. Notes tab to include comments on protocol **b.** 3D view of sample based on grey scale