QUEEN'S UNIVERSITY BELFAST

Faculty of Medicine Health and Life Sciences
Advanced Imaging Core Technology Unit (CTU)

Induction workbook

Content

1. HEALTH AND SAFETY

- The Advanced Imaging CTU is located in the basement of The Welcome-Wolfson Institute for Experimental Medicine building. Door OB.035, rooms OB.036-OB042.

- If you hear a fire alarm, please follow the **EXIT** signs and evacuate the building using the closest route.

- All users of the Advanced Imaging CTU are required to complete relevant Health and Safety, Laser Safety and Biosafety training prior to use of the facility. Users must abide by the Health and Safety guidelines of Queen's University Belfast and the Welcome-Wolfson Institute for Experimental Medicine. Please discuss relevant Health and Safety training courses with the CTU Manager.

  Guidelines on Health and Safety at Queen's University Belfast are available below:

http://www.qub.ac.uk/directorates/HumanResources/OccupationalHealthandSafety/GuidanceNotes/SafeWorkinginLaboratories/

http://www.qub.ac.uk/directorates/HumanResources/OccupationalHealthandSafety/GuidanceNotes/LaserSafety/

Safety signs related to the presence of Class 3 and 4 lasers and other possible hazards in the Advanced Imaging CTU are posted on the doors, walls of the rooms and microscopes as necessary. All the lasers are housed within an enclosed environment (black or white chambers) with interlocks, so that users are not exposed to the laser beams.

Risk Assessments for each instrument are provided, and located in the **red folder** in the microscope rooms. During the training, you will learn about the potential risks of using the microscopes and how to avoid them and use the equipment safely. Please read the Risk assessments for each microscope carefully.

- Users must have successfully complete or will take, the LIMITS LASER safety training course organised by CEM (Liza Colhoun).
-Gloves are **NOT** permitted to touch the microscopes and/or the computer keyboards/mice.

- Phones are located in the majority of the microscope rooms and the main corridor. In the case of emergency, please use the phone and dial the necessary phone number. Emergency numbers are posted next to the phones.

- Emergency Number Extension: 2222 (External 028 9097 2222)
- Internal Extension Numbers 5099 and 5098 (External 028 9097 5099)

- Accidents are very rare. In the event of an accident notify a member of staff so the appropriate action and reporting of the incident can be followed.

- No food or drink is allowed in the microscope rooms.

- If you need to work after 7pm or weekends, please let us known and fill out:

  - Lone/out of hours permit to work, attached to this workbook.

  - The sign in/out book at CEM reception of the WWIEM building must be completed so that Queen's security will know that you are working in the Advanced Imaging Unit CTU.

Internal Extension Numbers **5099** and **5098** (External 028 9097 5099)

- Colleagues or visitors are not authorised to operate equipment in any circumstances if they have not been trained by the Advanced Imaging CTU staff.

2. WASTE DISPOSAL

If you use chemicals that present a health hazard and require special disposal or manipulation, please let CTU staff know so we can provide proper protection and disposal.

- For gloves, hazardous waste and tissue culture dishes, please use the yellow bin provided in the room.
- For Hazardous waste like glass, sharp objects or needles please use the orange bins provided.
- For general waste like tissue lens tissue, use the general waste/rubbish disposal (white bins) provided in each microscope room
- Any Biohazardous waste should be taken back to the laboratory for proper disposal according to the Manufacturer Data Sheets (MSDS).
- Tissue, mice and cages (if applicable) must be taken back to laboratory for proper disposal.

3. DATA STORAGE AND TRANSFER

Images acquired while using the microscopes should be transferred to your computer via the network. Each computer network has its own IP address.

- EVOS IP: You need to bring your external hard drive to use it.
- FLEXSTATION3 Remote access - computer IP = \\143.117.119.174
- Leica DM5500 Fluorescence microscope- computer IP = \\143.117.119.226
- Nikon 6D live imaging computer IP: = \\143.117.119.179
- Nikon C1 you need to bring your external hard drive to save your experiments and transfer your data.
- SP5 confocal microscope. Remote access - computer IP = \\143.117.118.117
- SP8 confocal microscope. Remote access - computer IP = \\143.117.118.171
- SP8-MP multi photon microscope. Remote access - computer IP = \\143.117.119.166
- JEOL –Transmission Electron Microscope-we provide external hard drive
- IMARIS computer. Remote access – computer IP = \\143.117.119.217 if asked for log-in use ADS credentials.

To remote access a microscope computer from your own computer: go to my computer or Network and in your browser bar type **IP address** of the computer in the AI_CTU you want to access, press **Enter**

A box will appear asking for username and password (Leica computers)

Username = tcs-user then password will be either sp5, sp8 or sp8-mp

Password sp8-mp (lower case)

This will bring up the 'D' drive icon in the computer that you used for imaging

You can then access your data.

You can also use QUB Dropbox to transfer your data.

!!!! As a general rule, we can store your data on the microscope PC computer that you used to record your images until "D" drive storage reaches its limit. There is insufficient disk space to store all the data that users generate, so please transfer your data to your own PC as soon as possible after recording and delete your files from the microscope computer.

- ✓ When the microscope computer "D" drive storage is nearing its limit we will send an email so you have a chance to transfer your data before we have to delete data to free up storage space.

4. MICROSCOPE BOOKING

We are using **Faces Scheduling System** to book the microscopes (http://faces.ccrc.uga.edu/). After the training, the AI_CTU staff will send you an email invitation to join the Faces Scheduling System. You will get an automatic password and a username. You must change the temporary password and log in into Faces within 24 hours.

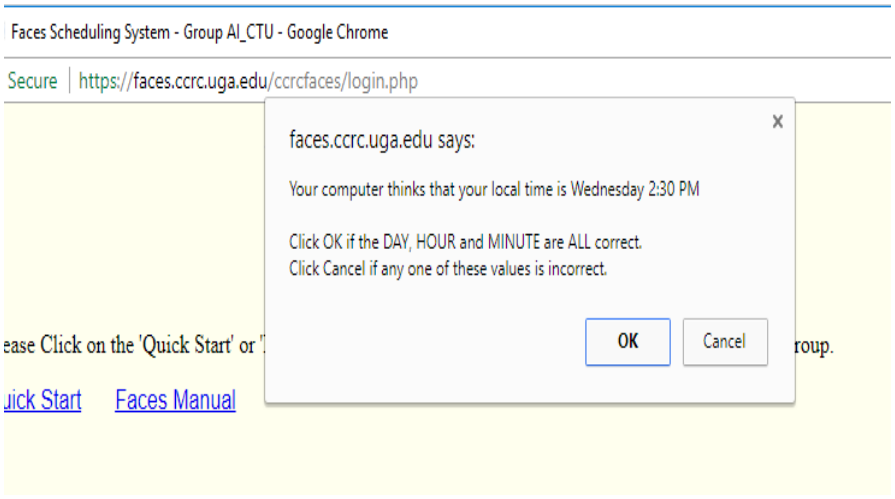
As a rule, you can book as many hours as you need to complete your imaging. However, if you have to cancel the time for different reasons, please let us known as soon as possible (ideally 12-24h beforehand), so other people can use the time. Users will be charged a £15 cancellation fee if they fail to cancel a booking that they later do not attend.

In the Faces Scheduling System, users can book the time to use a microscope after they been trained by the AI-CTU imaging team.

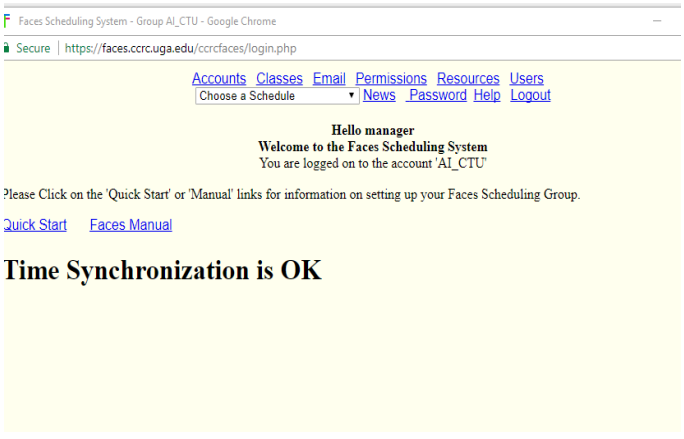
USER GUIDE:

Type in your browser: https://faces.ccrc.uga.edu/

Once you log in into Faces you will be asked if the local time is correct:



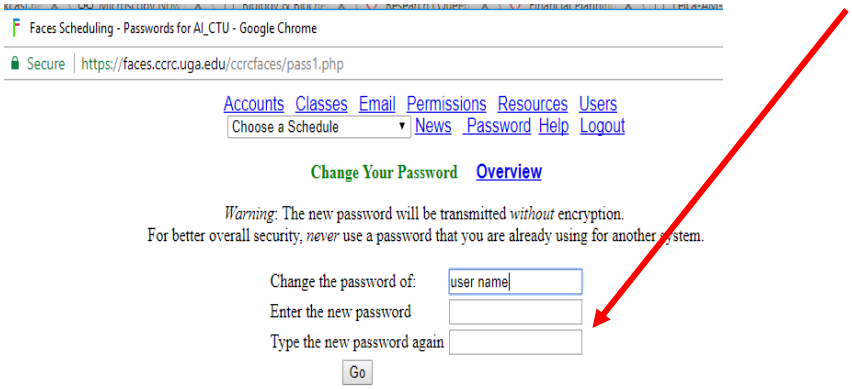
If it is correct, press OK. Then you will get this message.



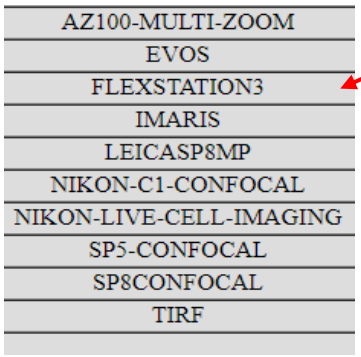
Time Synchronization is OK

Faculty of Medicine Health and Life Sciences
Advanced Imaging Core Technology Unit (CTU)

We recommend that you change your password at first login: You need to press **Password:**

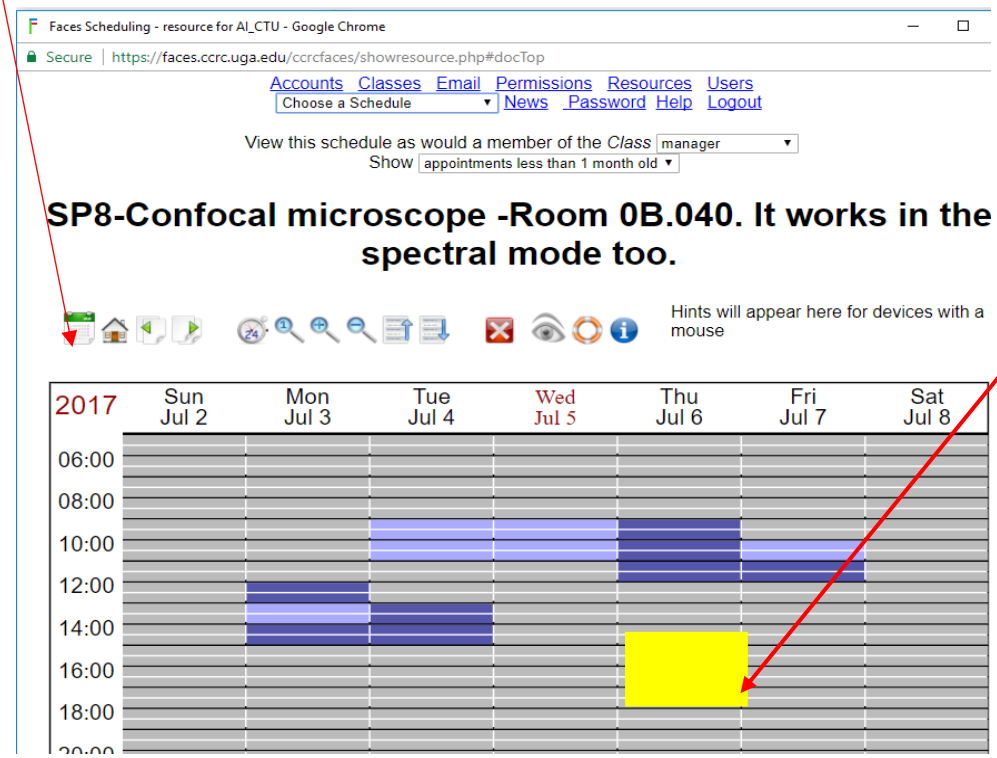


To book the microscope, you need to go to: Choose a Schedule, scroll down with the mouse and choose the microscope

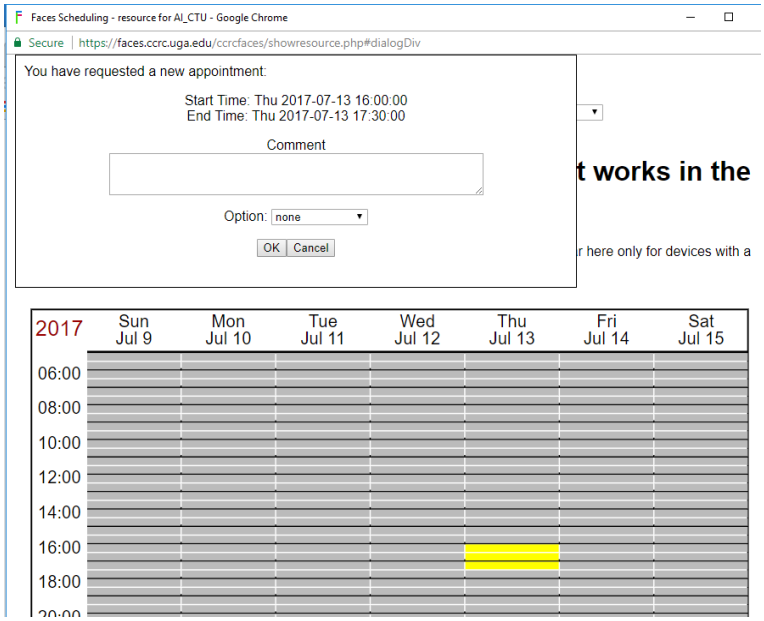


Click the calendar icon to choose a date

Click a time slot to select appointment start, then click another time slot to select the appointment end. The selected time will be highlighted in yellow

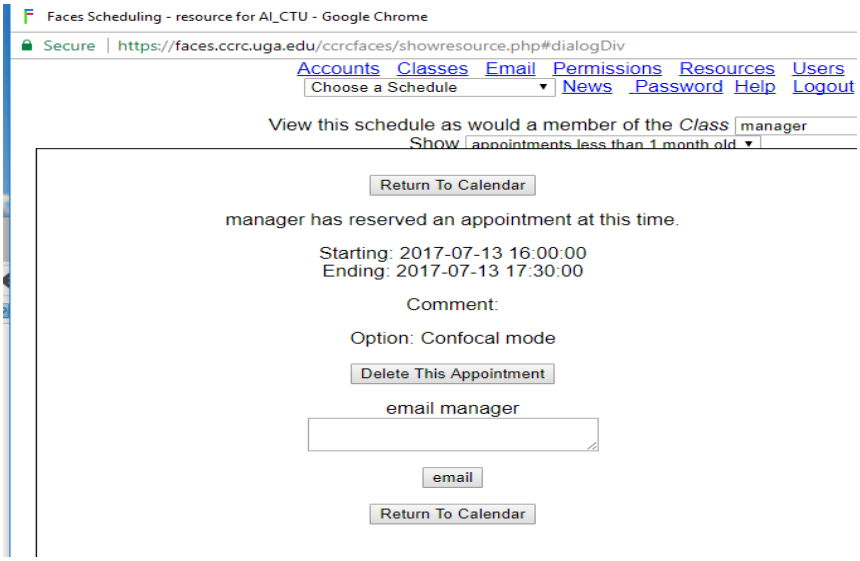


A window will appear to confirm your booking:



Then click OK:

If you want to delete the appointment Click on:



Definition of icons:

- Click the calendar to change the week displayed.
- Click the home icon to show the week containing today's date.
- The green left and right arrows move to the previous and next week.
- Click the 24 hour clock to see the full 24 hours.
- Click the restore view icon to reset default time range.
- Click the magnifiers to zoom the time range in or out.
- The blue up and down arrows move to earlier and later in the day.
- Click the red X to cancel the selection.
- Click the eye to toggle Color Blind Mode.
- Click the life saver to see this message.
- Click the information icon to learn about Faces.

5. INSTRUMENTS

- **Transmission Electron Microscope (TEM) –JEOL –JEM 1400Plus**

Can be used to image processed samples. The TEM is used to image cell and tissue ultrastructure.

Resolution of the TEM is 0.32 nm in point image and 0.2 nm in lattice image mode.

To obtain a lattice image, a large objective aperture has to be selected that allows many beams including the direct beam to pass. The image is formed by the interference of the diffracted beams with the direct beam (phase contrast).

!!Using the electron microscope does not pose any hazard. However, it generates a magnetic field that may be hazardous for a person with pacemaker or other electronic implant-devices.

To operate the JEM 1400 PLUS electron microscope, you must first be trained by one of the imaging core staff and follow the procedure described in the user manual attached.

Please refer to user manual for detailed information of how to use this microscope.

- **Widefield Fluorescence Microscopes**

    - **1. Leica Fluorescence Upright Wide-field Microscope DM5500**

QUEEN'S UNIVERSITY BELFAST

Faculty of Medicine Health and Life Sciences

Advanced Imaging Core Technology Unit (CTU)

Capabilities

- **Immunofluorescence, fixed samples, cell culture**
- **Imaging fluorophores from 400nm to 647 nm (near UV to Far Red)**
- **Transmission: phase contrast and polarization**

Equipment:

- **Leica DM5500B**
- **Leica EL6000 external light source (wide spectrum metal halide lamp)**
- **2 cameras: colour for brightfield and monochrome for fluorescence**
- **Software: LAS X**

Objective:

Magnification	NA	TYPE	Immersion medium	Working Distance (mm)	Cover glass	No
2.5x	**0.07**	PL FLUOTAR	**Dry**	**9.4**	**-**	**5670…**
5x	**0.15**	HCxPL FLUOTAR	**Dry**	**12.0**	**-**	**506224**
10x	**0.3**	HCPL Fluotar, Ph1	**Dry**	**11.0**	**-**	**506507**
20x	**0.5**	PL FLUOTAR-Ph2	**Dry**	**1.15**	**0.17**	**506506**
40x	**0.85**	HCxPL APO, CORR	**Dry**	**0.21**	**0.11-0.23**	**506294**
100X	**1.4**	HCxPL APO	**OIL**	**-**	**-**	**506220**

Filter cubes for eyepiece visualisation

Cube	Fluorophore	Excitation wavelengths	Dichroic	Excitation wavelength
A4	**DAPI**	**BP360/40**	**400**	**BP 470/40**
L5	**Alexa488, GFP,FITC**	**BP 480/40**	**505**	**BP 527/30**
TX2	**Alexa568, RFP, TRITC, TxRed**	**BP 560/40**	**595**	**BP 645/75**
Y5	**Alexa647, APC, Cy5**	**BP 620/60**	**660**	**BP 700/75**

!!!How to use it: Please refer to user manual for detailed information

1. **Log on to the computer**
2. **Switch the microscope on**
3. **Light source for fluorescence ON (** not required for brightfield)
4. **Load software**

- **2. Nikon Multi Zoom AZ 100 Multi-purpose Microscope**

Epi-fluorescence, diascopic Normarski DIC configuration

It is a stereoscopic widefield microscope with long working distance, and a biological microscope boasting high –resolution images.

Magnification from 5x to 400x, can switch from macro to micro observation of the same sample. This enables imaging of full tissue sections at the lowest magnification moving to the single cell level at the highest magnification.

Capabilities

- **Immunofluorescence, fixed samples, cell culture,**
- **Imaging fluorophores from 400nm to 594 nm (near UV to Red)**
- **Transmission: phase contrast and DIC polarization**

Equipment:

- **Nikon microscope**
- **Ds-Ri2Colour Camera**
- **C-HGFIE Intensilight HG Precentered Fibre Illuminator**
- **Wide spectrum metal halide lamp**
- **Software: NIS-Elements AR**

Objective

Magnification	NA	Type	Working distance (mm)	Number
0.5x	0.05	AZ-Plan Apochromat	54	MNH85050
1x	0.1	AZ-Plan Apochromat	34	MNH85100
5x	0.5	AZ-Plan Fluor	15	MNH85500

Filter cubes for eyepiece visualisation

Cube	Fluorophore	Excitation wavelengths	Dichroic	Excitation wavelength
C-FL EPI-FI	BFP	EX340-380	DM400	BA 435-485
C-FL EPI-FI	FITC	EX 465-495	DM505	BA 515-555
C-FL EPI-FI	TRITC	EX 540/25	DM565	BA 605/55

!!!How to use it: Please refer to user manual for detailed information

1. **Turn on the power supply**
2. **Turn on the microscope**
3. **Log on to the computer**

Turn on the Software: NIS-Elements AR

- ## 3. Nikon 6D Live image Cell Imaging Microscope

Capabilities

- **Immunofluorescence, fixed samples and live cell imaging.**

- **Imaging fluorophores from 400nm to 647 nm (near UV to Far Red)**
- **Transmission: phase contrast and DIC polarization**
- **Imaging of cell culture in multi-well plates in time in different points within a controlled environment**
- **Time lapse imaging**
- **Fast image acquisition**

Equipment:

- **Nikon Eclipse Ti-E microscope**
- **PFS-S Perfect focus unit with motorised nose piece**
- **Lumencor SPECTRAX CHROMA illumination unit**
- **Andor sCMOS Camera**
- **Software: NIS-A Elements for imaging and analysis**
- **Active CO2 and O2 (Hypoxia) controller**
- **OKO-Touch Temperature control unit and CO2**
- **Perfusion system & rapid drug changer VC-8P and VC-77SP8E**

Objective:

Magnification	NA	TYPE	Immersion medium	Working Distance (mm)	Cover glass mm	No
40x	**1.3**	CFI60 Plan FLUOR	**oil**	**0.2**	**-**	**MRH01401**
20xC	**0.45**	CFI60 Super Plan Fluor ELWD	**dry**	**6.9-8.2**	**0-0.2**	**MRH08230**
40xC	**0.6**	CFI60 Super Plan Fluor ELWD	**dry**	**2.8-3.6**	**correction ring**	**MRH08430**
10x	**0.13**	CFI60 Plan Fluor DLL	**dry**	**16 PhL**		**MRH10101**
100x	**1.45**	CFI60 Plan Apochromat Lambda	**oil**	**0.13**		**MRD01905**
20x	**0.75**	CFI60 Plan Apochromat VC	**dry**	**1.00**		**MRD70200**
60x	**1.4**	CFI60 Apochromat Lambda S	**oil**	**0.14**		**MRD71600 Chromatic Correction 405-656 nm**

Filter cubes for eyepiece visualisation

Cube	Fluorophore	Excitation wavelengths
1	DAPI, FITC, Cy3	405, 470,630
2	CFP, YFP, Tx red , far red	385, 505, 525, 560,625

!!!How to use it: Please refer to user manual for detailed information

1. **If you need to use the temperature and CO_2 controller, you need to put this equipment on first. It takes around 30 min to reach the desired CO2 and temperature level**
2. **Switch on the Lumencor , the light source for fluorescence**
3. **Turn on the camera**
4. **Turn on Software NIS**

• 4. EVOS FL Auto 2 Imaging System

Capabilities

- **Immunofluorescence, fixed samples, cell culture, multi-well plates**
- **Imaging of Fluidigm single cell genomics chips**
- **Imaging fluorophores from 400nm to 580 nm (near UV to Red)**
- **Transmission: phase contrast**
- **Long time, Time lapse imaging**
- **Environmental control-** onstage incubator that enables precise control of temperature, humidity and CO2
- **Image stitching**

Equipment:

- **Microscope**
- **Camera**
- **Environmental control chamber**
- **Software: EVOS AutoFL**

Objective

Magnification	NA	Type	Working distance (mm)	Number
10x	0.25	AMG LPlan FL PH	9.20	AMEP-4681
20x	0.4	AMG LPlan FL PH	3.1	AMEP-4682
4x	0.13	AMG-LPlan FL PH	16.9	AMEP-4680
40x	0.65	AMG LPlan FL PH	1.6	AMEP-4683
40x	0.75	Plan FL Cover Slip	2.2 Corrected for coverslip	AMEP-4699

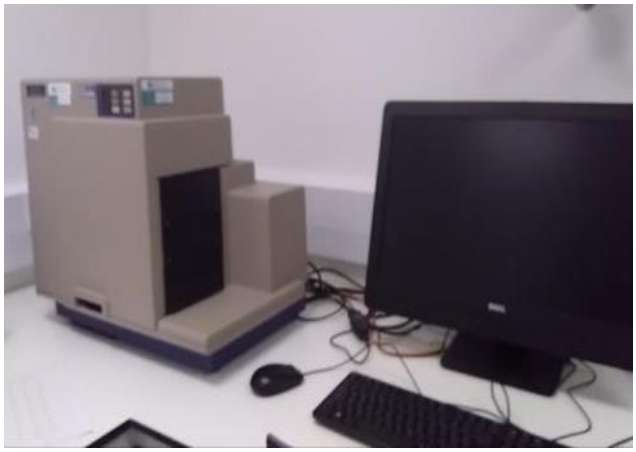
Filter cubes for visualisation

Cube	Fluorophore	Excitation wavelengths	Emission wavelength
1	DAPI	EX360	447 nm
2	GFP	EX 470	525
3	Texas Red	EX 530	593

!!!How to use it: Please refer to user manual for detailed information

1. **Turn on the power supply**
2. **Turn on the microscope**
3. **Log on to the computer**
4. **Turn on the Software : EVOS**
5. **You need an external hard drive to use it**

- **5. FLEXSTATION3**



Capabilities

- **Cell culture**
- **Screening fluorophores from 200nm to 1000 nm (UV to InfraRed)**
- **Microplate reader designed for a wide range of biochemical and cell-based high-throughput screening assays.**
- **96 or 384 well plates**
- **Programmable well-to-well drug delivery**
- **Environmental control** that enables precise control of temperature
- The two holographic diffraction grating monochromators allow selection
- of any wavelength between 200 nm and 1000 nm in absorbance;
- 250 nm and 850 nm in fluorescence intensity,
- time-resolved fluorescence (TRF), or luminescence ;
- and 400 nm and - 750 nm for readings in fluorescence polarization.

Please refer to user manual for detailed information on how to use this equipment.

Confocal microscopes

- **1. Leica SP5**

Capabilities

- **Immunofluorescence, fixed samples, cell culture**
- **Confocal imaging fluorophores from 400nm to 647 nm (near UV to Far Red)**
- **Transmission: phase contrast and DIC polarization**

Equipment:

- **Leica microscope TCS-SP5**
- **Software LAS AF for imaging and analysis**
- **Supply unit**
- Acousto-optic tuneable filter (AOTF)

Objective:

Magnification	NA	TYPE	Immersion medium	Working Distance	Cover glass mm	No
40x	1.25	HCX PL APO, PH3	oil	1	0.17	11506106
20x	0.5	HC PL Fluotar	dry	1.1	0.17	11506506
40x	0.75	HCxPL Fluotar	dry	0.4	0.17	11506145
100x	1.4	HCxPL APO	oil	-	0.17	11506220
20x	0.7	HCxPL APO lambda blue IMM UV	dry	2.6	UV, 405 correction optic	11506191
63x	1.4	HCX PL APO lambda blue	oil	-	UV, 405 correction optic	11506192

Lasers and filter cubes for visualisation. This system - has a spectral detector, so almost all the dyes can be visualized.

Laser	Fluorophore	Excitation wavelengths	Emission wavelength
405 Blue Diode	DAPI		Spectral detector
Argon	Cyan, FITIC, YFP, GFP, Alexa 488, Cy2	458, 476, 488, 496, 514	
DPSS-Diode	Alexa 568, TRITC, Cy3	561	
Helium Neon-Gas laser	Alexa 594, Texas Red, mCherry	594	
Helium Neon-Gas laser	Alexa 633, Alexa 647, CY5	633	

!!!How to use it: Please refer to user manual for detailed information

1. **Turn on the lasers , the controller and the light source for fluorescence**
2. **Turn on the software LAS AF**

2. Leica TCS SP8-Spectral Confocal Microscope

Capabilities

- **Immunofluorescence, fixed samples, cell culture, multi- well plates**
- **Imaging fluorophores from 400nm to 647 nm (near UV to Far Red)**
- **Transmission: phase contrast and DIC polarization**
- **Spectral confocal detection with HyD GaAsP detectors**
- **Imaging of cell culture in multi-well plates in time within a controlled environment**
- **Fast imaging acquisition 100 frames/s using fast resonant scanner**
- **Motorised XY scanning stage for multi-point tiled imaging**
- **Deconvolution module to increase optical resolution**

- **Microscope Temperature, humidity , CO2 active unit and perfusion unit**

Equipment:

- **Leica TCS SP8 microscope**
- **Anti-vibration table**
- **EL600 Fluorescence illumination unit**
- **Software: LAS X for imaging and analysis**
- **Perfusion system & rapid drug changer VC-8P and VC-77SP8E**

Objective:

Magnification	NA	TYPE	Immersion medium	Working Distance (mm)	Cover glass mm	No
10x	**0.3**	HC PL FLUOTAR good colour correction	**dry**	**11**	**-**	**15506505**
20x	**0.75**	HC PL APO CS2 Superior Colour correction for confocal scanning	**dry**	**0.62**	**0.17**	**15506517**
40x	**1.10**	HC PL APO W CORR CS2 superior colour correction, optimized for confocal scanning applications	**water**	**0.65**	**0.14-0.18**	**15506357**
40x	**1.25**	HCX PL APO, PH3	**oil**	**-**	**0.17**	**11506106**
63x	**1.4**	**HCX PL APO lambda blue**	**oil**	**-**	**UV, 405 correction optic**	**11506192**
100x	**1.4**	HC PL APO CS2	**oil**	**0.13**	**0.17**	**15506372**

Lasers and filter cubes for visualisation

Lasers	Fluorophore	Excitation wavelengths	Dichroic	Emission wavelength
405 nm	DAPI long pass	BP 360/40		LP 425
488 nm	FITIC LP	BP 470/40	LIAchroic beam splitter for 448 / 514	LP 515
514 nm				
552nm	RHOD LP	BP 540/45		LP 590
638 nm				

!!!How to use it: Please refer to user manual for detail information

1. **If you need to use the temperature and CO_2 control controller, you need to put this equipment on first. It takes around 30 min to reach the desired CO_2 and temperature level**
2. **Switch the LASER on , and the light source for fluorescence metal halide lamp on**
3. **Turn on Software LAS-X**

3. Leica SP8-Upgright installed on MP

Capabilities

- **Immunofluorescence, fixed samples, cell culture, live tissue**
- **Imaging fluorophores from 400nm to 647 nm (near UV to Far Red)**
- **Transmission: phase contrast and DIC polarization**
- **Fast imaging acquisition 100 frames/sec**

Equipment:

- **Leica SP8 Confocal microscope upright fixed stage**
- **Transmitted light bright field detector**
- **LASERS**
- **Metal halide wide spectrum fluorescence lamp**

- **Software: LAS X for imaging and analysis**
- **Active CO2 and O2 (Hypoxia) controller**
- **Perfusion system & rapid drug changer VC-8P and VC-77SP8E**

Objective:

Magnification	NA	TYPE	Immersion medium	Working Distance (mm)	Cover glass mm	No
10x	**0.4**	HC PL APO, PH1		**2.2**	**0.17/A**	**15506286**
10x	**0.3**	HCX APO L W excellent colour correction and high transmission	**water**	**3.6**		**15506142**
20x	**0.5**	HC PL FLUOTAR	**air**	**1.15**	**0.17**	**15506503**
25x	**0.95**	HC FLUOTAR L W VISIR intravital imaging; High transmission >83% from 4001300 nm. Colour corrected for VIS and NIR up to 950 nm.	**water**	**2.5**		**15506374**
40x	**0.6**	HCX PL FL L CORR PH2 02/ C,Obj.	air	**3.3-1.9**	**0-2 with correction collar**	**15506203**
40x	**0.85**	HCX PL APO CORR CS,0.11 superior colour correction	**air**	**0.21**	**0.11-0.23**	**15506295**
63x	**1.2**	HC PL APO W CORR CS2	**water**	**0.3**	**0.14-0.18**	**15506346**
63x	**0.9**	HC APO L W UVI CS2	**water**	**2.2**		**15506362**

Lasers and Filter cubes for visualisation

Lasers	Fluorophore	Emission Wavelength
405	DAPI,	
488	YFP	
514		
552		
638 nm		

If you need to use the temperature and CO_2 control, you need to put this equipment on first. It takes around 30 min to reach the desired CO_2 and temperature level

Switch on the LASERS,

Turn on the HYBRID detectors chiller

Turn on Software LAS X

4. Nikon C1

Capabilities

- **Immunofluorescence, fixed samples, cell culture**
- **Imaging fluorophores from 400nm to 594 nm (near UV to Red)**
- **Transmission: phase contrast and DIC polarization**

Equipment:

- **Nikon Eclipse Ti-E microscope**
- **Software: Easy-C1 for imaging / NIS elements**

Objective:

Magnification	NA	TYPE	Immersion medium	Working Distance (mm)	Cover glass mm
40x	0.75	Plan FLUOR	dry	0.72	
20x	0.45	Plan Fluor ELWD	dry		
10x	0.3	Plan Fluor	dry	16	-
40x long working distance	0.6	S Plan Fluor	dry	3.6-2.8	

(*) 60x water objective lens available on request

Lasers and filter cubes for visualisation

Laser	Fluorophore	Emission wavelengths	Dichroic
L1 405 diode laser	DAPI,		
L2 488 nm diode laser	YFP		
L3 561 diode laser	RHOD		

!!!How to use it: Please refer to user manual for detail information

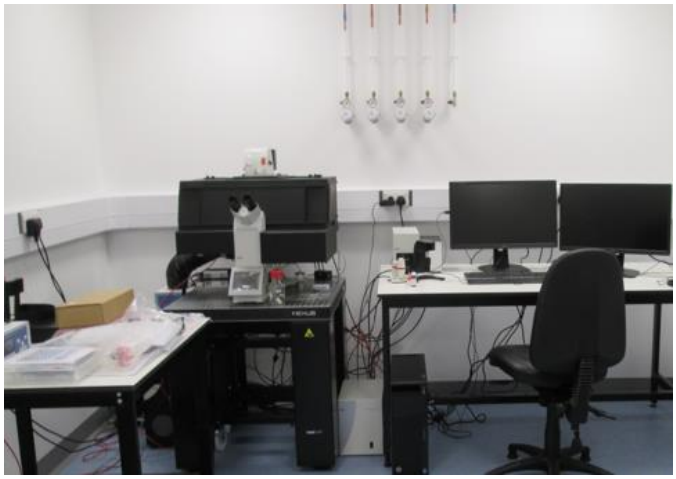
1. **Switch on Sapphire 488 laser**
2. **Switch on DPSS-Melles Griot laser unit**
3. **Turn on Blue laser**
4. **Turn on Epi-fluorescent bulb and ignition on the burner**
5. **Switch on bright light bulb source**
6. **Turn on RFA unit and Controller- Eclipse-C1 UNIT**
7. **Open EZ-C1 3.6 software**

QUEEN'S UNIVERSITY BELFAST

Faculty of Medicine Health and Life Sciences

Advanced Imaging Core Technology Unit (CTU)

d. TIRF –Leica-Total Internal Reflection



Capabilities

- **Cell culture on special Petri dish**
- **Imaging fluorophores from 400nm to 635 nm (near UV to Far Red)**
- **Fast imaging acquisition 100 frames/sec**
- **Differential Interference Contrast**
- **Visualize ultra-fast events like Ca^{2+} sparks, puffs**
- Visualize and measure interactions of single molecules, the kinetics or co-localization of molecules

Equipment:

- **DMi8 with TIRF Multi Colour microscope**
- **Andor ZYLA 4.2 sCMOS Camera**
- **High precision z-focus for parafocalty of all objectives and long-time stability**
- **Advanced condenser S28/N.A. 0.55**
- **Anti - vibration table**
- **Condenser base 1-28 f. fixed lens, motor (free working distances 1-28 mm, field of view 25 mm (BF, PH, DF, Pol, DIC,IMC)**
- **TIRF module**
- **Alignment camera GIST/TIRF**
- **External light source EL6000**
- **Software: Leica –LAS X for imaging and analysis**
- **Incubator i8 for TIRF- large black environmental chamber with safety interlock**
- **Perfusion system & rapid drug changer VC-8P and VC-77SP8E**
- **Temperature control unit Temp Controller 2000-2 with two independent channels**
- **Heating unit 2000 supplies the large Leica incubators with heated air up to 30C.**
- **CO2 controller 2000 with concentration 0-20 Vol%**
- **Heated mounting frame**
- **POC-R2 cell cultivation system**

- **Humidifier PM**

Objective:

Magnification	NA	TYPE	Immersion medium	Working Distance (mm)	Cover glass mm	No
10x	0.4	HC PL APO	dry	2.2	0.17 DIN/ISO	11506284
20x	0.7	HC PLAN APO	dry		0.17/C 0.59	11506166
40x	0.85	HC PL APO	dry	0.21	0.11-0.23	11506294
63x	1.47	HC PL APO Optimized for TIRF	oil	0.1	0.1-0.22	11506319
100x	1.47	HC PL APO Optimized for TIRF	oil	0.09	0.1-0.22	11506318

Lasers and filter cubes for visualisation

Laser	Fluorophore
405	DAPI
488	YFP
561	
635	

Filter cubes

Filter cube name	Excitation wavelength	Dichroic	Emission wavelength
GFP for TIRF, GFPT			
CFP for TIRF, CFPT			
Y3 for TIRF, Y3T	555/25	575	605/55
Y5 for TIRF, Y5T	630/60	660	700/75
QUAD for TIRF, QUADT	405/10 , 488/13; 561/10; 635/15	418, 595, 570, 655	450/55; 525/50; 605/45; 730/100

!!!How to use it: Please refer to user manual for detailed information

1. If you need to use the temperature and CO_2 controller, you need to put this equipment on first. It takes around 30 min to reach the desired CO_2 and temperature level

2. You need to seed/culture cells on # 1.5 cover glass (thickness 0.17) ;). These slides can be purchased from:

http://ibidi.com/xtproducts/en/ibidi-Labware/Open-Slides-Dishes:-Glass-Bottom/m-Dish-35-mm-high-Glass-Bottom

www.eppendorf.co.uk or Angela Lombard territory manager: lombard.a@eppendorf.co.uk

3. **Switch on the LASERS**
4. **Turn on the camera, stage controller, camera**
5. **Turn on LAS X Software**

Leica SP8-MP (Multiphoton excited fluorescence microscope)

Multiphoton microscope- Leica SP8

Multiphoton excitation microscopy is a nonlinear event that employs two or more photon with pulsed IR high power laser to excite a fluorophore. Because of the low energy of the IR laser beam it can penetrate deeper within the tissue and generate less scattering, photo-bleaching or photo-destruction.

Capabilities

- **Imaging fluorophores from 300nm to 800 nm (near UV to Far Red)**
- **Fast imaging acquisition 100 frames/sec**
- **Differential Interference Contrast**
- **Visualize ultrafast events like Ca^{2+} sparks, puffs**
- Visualize and measure interactions of single molecules, the kinetics or co-localization of molecules
- Image fluorophore in deep in tissue, cell culture , *in vivo animals* or *in vitro*
- Fluorescence lifetime imaging module
- Polarization control
- Spectral imaging

Equipment:

- **DM6000CFS fixed stage Upright Microscope with both 2 and 6 position nosepiece and XY Motorised stage and Super Z-Galvo Fast focusing stage-** with working distances adjustable for microscope slides and small animal in vivo imaging equipped with fixed stage-DM6000FS-that can accommodate whole tissue, animal imaging, slides and Petri dish
- **Anti-vibration table-1200x1500 Optical Table with Air compressor**
- **Scan head**

- Epifluorescence unit – EL6000 extended life FL unit .Fibre coupled, alignment free HXP 120 metal halide fluorescence light source with motorised attenuation. Fluorescence filters for blue, green and red excitation

- **Fast Resonance Scanning capability with Tandem Scanner** Imaging: High Resolution (3,6kHz) + **Fast (8kHz) Resonant Scanner, 29fps , 512x512 full field zoom 1.7**

- Software: Leica –LAS X for imaging and analysis
- Incubator i8 for TIRF- large black environmental chamber with safety interlock
- Perfusion system & rapid drug changer VC-8P and VC-77SP8E
- **Multi-photon laser: Mai Tai eHP DeepSee IR laser excitation** from **690-1040nm**, pre-chirped/short pulse width for compensation with deep tissue imaging- high performance / low scattering.
- EOM (Electro Optical Modulator) Controller
- HyD hybrid detectors power and cooling unit
- Beam routing optics and coupling unit for fast IR laser attenuation
- Mai Tai laser power supply and CW diode pumped laser solid state 532 nm
- Chiller - ThermoRack 401 (Nalco 460-PCCL104 liquid corrosion inhibitor as a coolant. Do not use deionized water) for cooling IR laser

- **RLD IR Detectors:**

- x1 Channel TLD Transmitted Light Detector for IR brightfield and SHG imaging in the forward direction.

- X2 HyD GaAsP Detectors Plus x2 PMT RLD detectors for IR imaging with high sensitivity, low scattering. Expandable with additional x1 2Ch HyD as required.

- RLD HyD filter blocks are included for FITC/TRITC, SHG, CFP_YFP and an empty filter to build your own combination filter, for imaging in backward direction a FITC/TRITC block and SHG block are also included.

QUEEN'S UNIVERSITY BELFAST

Faculty of Medicine Health and Life Sciences
Advanced Imaging Core Technology Unit (CTU)

Objective:

Magnification	NA	TYPE	Immersion medium	Working Distance (mm)	Cover glass mm	No
10x	**0.4**	HC PL APO, PH1	**dry**	**2.2**	**0.17/A**	**15506286**
10x	**0.3**	HCX APO L W excellent colour correction and high transmission	**water**	**3.6**		**15506142**
20x	**0.5**	HC PL FLUOTAR	**dry**	**1.15**	**0.17**	**15506503**
25x	**0.95**	HC FLUOTAR L W VISIR intravital imaging; High transmission >83% from 400-1300 nm. Colour corrected for VIS and NIR up to 950 nm.	**water**	**2.5**		**15506374**
40x	**0.6**	HCX PL FL L CORR PH2 02/ C,Obj.	**dry**	**3.3-1.9**	**0-2 with correction collar**	**15506203**
40x	**0.85**	HCX PL APO CORR CS,0.11 superior colour correction	**dry**	**0.21**	**0.11-0.23**	**15506295**
63x	**1.2**	HC PL APO W CORR CS2	**water**	**0.3**	**0.14-0.18**	**15506346**
63x	**0.9**	HC APO L W UVI CS2	**water**	**2.2**		**15506362**

!!!How to use it- Please refer to user manual for detailed information

- **You need to turn on first the chiller for HyD detectors**
- **You turn on all the lasers and controller**
- **Turn on LAS-X software last**

6. Software for Image analysis available :

- **LEICA –LAS X : http://www.leica-microsystems.com/products/microscope-software/**
- **IMARIS-Bitplan: http://www.bitplane.com/imaris/imaris**
- **NIS-Elements : https://www.nikoninstruments.com/Products/Software**
- **Cell –Imaging software for Life Sciences Microscopy : Olympus Soft Imaging solution GmbH**
- **SoftMax-Pro software for analysis FLEXSTATION experiments**
- **http://cellprofiler.org/**
- **IMAGEJ –FIJI : https://imagej.net/Fiji/Downloads**

7. Useful references and links to microscopy websites

- -https://www.microscopyu.com/techniques/multi-photon/multiphoton-microscopy
- http://www.ammrf.org.au/myscope/confocal/confocal/image/seqsim/
- https://www.thermofisher.com/uk/en/home/life-science/cell-analysis/labeling-chemistry/fluorescence-spectraviewer.html#
- https://www.leica-microsystems.com/science-lab/
- http://www.ammrf.org.au/myscope/
- http://www.thermofisher.com/uk/en/home/life-science/cell-analysis/labeling-chemistry/fluorescence-spectraviewer.html

- Zipfel, W. R., Williams, R. M., and Webb, W. W., Nonlinear magic: Multiphoton microscopy in the biosciences., *Nature Biotechnology* 21: 1369-1377 (2003).
- https://micro.magnet.fsu.edu/primer/techniques/fluorescence/multiphoton/multiphotonintro.html
- Hoover E.E. & Squier J.A., Advances in multiphoton microscopy technology *Nature Photonics* 7, 93–101(2013)doi:10.1038/nphoton.2012.361
- Helmchen F. & Denk W., Deep tissue two-photon microscopy. *Nature Methods* - 2, 932 - 940 (2005)
- Svoboda K., and Yasuda R., Principles of Two-Photon Excitation Primer Microscopy and Its Applications to Neuroscience. *Neuron* 50, 823–839, June 15, 2006
- Pawley, James, ed. 1995. *Handbook of Biological Confocal Microscopy. 2nd edition*. Plenum Press, New York
- Joseph R Lakowicz, 2007. Principles of fluorescence spectroscopy, Springer

Contact information:
Dr Ileana Micu, Advanced Imaging CTU Manager: I.Micu@qub.ac.uk
Mrs Andrena Millar, Advanced Imaging CTU Technician: a.millar@qub.ac.uk
Telephone: +44 (0)28 9097 6120 / QUB internal x 6120
Professor Tim Curtis, Advanced Imaging CTU Academic Lead: t.curtis@qub.ac.uk

Website: https://www.qub.ac.uk/sites/core-technology-units/AdvancedImaging/