LUDWIG INSTITUTE FOR CANCER RESEARCH

Centre for Advanced Microscopy

Equipment Available

Widefield Microscopes

- **Nikon SMZ-1500** – Stereo microscope with transmitted, reflected, darkfield and fluorescence illumination
    - Magnification range 0.75-11.25x
    - Fitted with Nikon DMX1200C colour camera
    - Uses Nikon NIS Elements F software for capture

Installed filters

Flurophore	Excitation	Dichroic	Emission
DAPI, Hoechst, BFP	470/40	495LP	515LP
GFP, FITC, Alexa 488	460/20	505LP	530/30
Rhodamine, Texas Red, DsRed	546/10	565LP	590LP

- **Nikon Eclipse 80i** –Upright compound microscope with brightfield (transmitted only) illumination
    - Fitted with Nikon DMX1200C cameras
    - Uses Nikon NIS Elements AR (v3.06) for capture

Installed objectives

Magnification	NA	Contrast Method	Working Distance (mm)	Series
4x	0.13	None	17.10	Plan Fluor
10x	0.30	Phase	16.00	Plan Fluor
20x	0.50	Phase	2.10	Plan Fluor
40x	0.60	Phase	3.7-2.7	Plan Fluor
60x (Oil)	1.40	DIC	0.21	Plan Apo

- **Nikon Eclipse 90i** – Fully motorised upright compound microscope with brightfield (transmitted, phase and DIC; currently equipped with DIC only objectives) and fluorescence illumination
    - Fitted with SPOT RT3 Slider (Diagnostic Instruments, Inc.) and Nikon DMX1200C cameras
    - Has motorised stage to allow montage image capture
    - Uses SPOT Advanced (v4.7.0.25) and Nikon NIS Elements AR (v3.06)for capture

Installed objectives

Magnification	NA	Contrast Method	Working Distance (mm)	Series
2x	0.06	None	7.40	Plan
4x	0.13	None	17.10	Plan Fluor
10x	0.30	DIC	16.00	Plan Fluor
20x	0.50	DIC	2.10	Plan Fluor
40x (Oil)	1.3	DIC	0.22	S Fluor
60x (Oil)	1.40	DIC	0.21	Plan Apo

Installed filters

Flurophore	Excitation	Dichroic	Emission
DAPI, Hoechst, Alexa 405	360/20	400LP	460/25
GFP, FITC, Alexa 488	480/15	505LP	535/20
Rhodamine, TRITC, Alexa 546	540/25	565LP	605/55
Texas Red, Alexa 564	560/20	595LP	630/30
Alexa 633, Alexa 647, Cy5	640/30	660LP	700/75

- **Nikon Eclipse TE300** – Inverted compound microscope with brightfield (transmitted and phase) and fluorescence illumination.
    - Fitted with Coolsnap FX (Photometrics, Inc.) camera
    - Uses µManager (v1.3.46) software for capture

Installed objectives

Magnification	NA	Contrast Capable	Working Distance (mm)	Series
4	0.13	Phase	16.40	Plan Fluor
10	0.30	Phase	16.00	Plan Fluor
20	0.45	Phase	7.4	Plan Fluor
40	0.60	Phase	3.7-2.7	Plan Fluor

Installed filters

Flurophore	Excitation	Dichroic	Emission
DAPI, Hoechst, Alexa 405	360/20	400LP	460/25
GFP, FITC, Alexa 488	480/15	505LP	535/20
Rhodamine, TRITC, Alexa 546	535/25	575LP	590LP

- **Nikon Eclipse Ti-U** – Inverted compound microscope with brightfield (transmitted and phase) and fluorescence illumination.
    - Fitted with Coolsnap HQ (Photometrics, Inc.) camera
    - Uses µManager (v1.3.46) software for capture

Installed objectives

Magnification	NA	Contrast Capable	Working Distance (mm)	Series
4	0.13	Phase	16.40	Plan Fluor
10	0.30	Phase	16.00	Plan Fluor
20	0.45	Phase	7.4	Plan Fluor
40	0.60	Phase	3.7-2.7	Plan Fluor

Installed filters

Flurophore	Excitation	Dichroic	Emission
DAPI, Hoechst, Alexa 405	350/25	400LP	460/25
GFP, FITC, Alexa 488	470/20	495LP	525/25
Rhodamine, TRITC, Alexa 546	560/20	585LP	630/35

- **Nikon Eclipse Ti-E** – Fully motorised inverted compound microscope with brightfield (transmitted, phase and DIC) and fluorescence illumination. Set up for live cell imaging.
    - Fitted with SPOT Pursuit Slider (Diagnostic Instruments, Inc.) camera
    - Uses SPOT Advanced (v4.7.0.25) and MetaMorph (v7.7.0.1) software for capture
    - Fitted with Prior ProScan II motorised stage for multiple position time-lapse imaging
    - Fitted with incubator and gas control for long term imaging
    - Fitted with fast (40ms) Uniblitz shutter to minimise exposure

Installed objectives

Magnification	NA	Contrast Capable	Working Distance (mm)	Series
4	0.13	Phase	16.40	Plan Fluor

10	0.30	Phase	16.00	Plan Fluor
20	0.50	Phase	2.10	Plan Fluor
40	0.60	Phase, DIC	3.70	Plan Fluor
60	0.95	DIC	0.15	Plan Apo

Installed filters

Flurophore	Excitation	Dichroic	Emission
DAPI, Hoechst, Alexa 405	360/20	400LP	460/25
GFP, FITC, Alexa 488	480/15	505LP	535/20
Rhodamine, TRITC, Alexa 546	540/25	565LP	605/55
Texas Red, Alexa 564	560/20	595LP	630/30
Alexa 633, Alexa 647, Cy5	640/30	660LP	700/75

Confocal Microscopes

- **Nikon C1+ Confocal** – Attached to Nikon Eclipse TE-300 inverted compound microscope with brightfield (transmitted, phase and DIC) and fluorescence illumination.
    - Filter based confocal system
    - 405nm, 488nm and 532nm excitation lasers
    - 3 PMTs for detection
    - AOM Control for ROI scanning/bleaching/activation
    - Fitted with incubator and gas control for long term imaging
    - Fitted with Prior nanodrive motorised focus attachment for Z series acquisition
    - Uses Nikon EZ-C1 (v3.80) software for capture

Installed objectives

Magnification	NA	Working Distance (mm)	Series
10	0.50	1.20	S Fluor
20	0.50	12.10	Plan Fluor
40 (Oil)	1.30	0.22	S Fluor
60 (Oil)	1.40	0.21	Plan Apo (VC)

Installed filters for visualisation down microscope

Flurophore	Excitation	Dichroic	Emission
DAPI, Hoechst, Alexa 405	360/20	400LP	460/25
GFP, FITC, Alexa 488	480/15	505LP	535/20
Rhodamine, TRITC, Alexa 546	540/25	565LP	605/55

Installed filters for confocal capture

Flurophore	Emission
DAPI, Hoechst, Alexa 405	450/35
GFP, FITC, Alexa 488	510/20
Rhodamine, TRITC, Alexa 546, Alexa 564, Alexa 594	605/75

- **Olympus FV1000 Spectral Confocal with Multiphoton Capability** – Attached to Olympus IX-81 inverted compound microscope with brightfield (transmitted and DIC) and fluorescence illumination. Has MaiTai DeepSee Ti:Sapphire laser attached for multiphoton imaging.
    - Spectral based detection system
    - Capable of lambda scanning and spectral unmixing
    - 405nm, 473nm, 562nm and 635nm lasers for single photon excitation
    - Tuneable (690-1020nm) Ti:Sapphire laser for multiphoton excitation
    - 3 internal PMTs, 1 Transmitted light detector, 2 external PMTs (non-de-scanned)
    - AOM control for ROI scanning/bleaching/activation
    - SIM scanner (using additional 405nm laser) for simultaneous bleaching/imaging experiments
    - Fitted with incubator and gas control for long term imaging
    - Uses Olympus FV10-ASW (v1.7c) software for capture

Installed objectives

Magnification	NA	Working Distance (mm)	Series
10	0.40	3.10	UPlanSApo
20	0.75	0.60	UplanSApo
40	0.90	0.18	UplanSApo
60 (Water)	1.20	0.28	UplanSApo
60 (Oil)	1.35	0.15	UplanSApo

Installed filters for visualisation down microscope

Flurophore	Excitation	Dichroic	Emission
DAPI, Hoechst, Alexa 405	357/27	400LP	420LP
GFP, FITC, Alexa 488	482/12	505LP	530/20
Rhodamine, TRITC, Alexa 546, Alexa 564, Alexa 594	540/10	570LP	600/25

- **Olympus FV1000 Spectral Confocal with Multiphoton Capability** – Attached to Olympus BX-61W upright compound microscope with brightfield (transmitted and DIC) and fluorescence illumination. Has MaiTai DeepSee Ti:Sapphire laser attached for multiphoton imaging.
    - Spectral based detection system
    - Capable of lambda scanning and spectral unmixing
    - 405nm, 473nm, 562nm and 635nm lasers for single photon excitation
    - Tuneable (690-1020nm) Ti:Sapphire laser for multiphoton excitation
    - 3 internal PMTs, 1 Transmitted light detector, 2 external PMTs (non-de-scanned)
    - AOM control for ROI scanning/bleaching/activation
    - Fitted with incubator and gas control for long term imaging
    - Fitted with anaesthesia machine (Isoflurane vaporiser) for whole animal imaging
    - Fitted with HEPA filtered extraction fan for removal of mouse dander and odour
    - Uses Olympus FV10-ASW (v1.7c) software for capture

Installed objectives

Magnification	NA	Working Distance (mm)	Series
20 (Water)	0.95	2.00	XLUMPlanFL
25 (Water)	1.05	2.00	XLPlanN

Installed filters for visualisation down microscope

Flurophore	Excitation	Dichroic	Emission
DAPI, Hoechst, Alexa 405	357/27	400LP	420LP
GFP, FITC, Alexa 488	482/12	505LP	530/20
Rhodamine, TRITC, Alexa 546, Alexa 564, Alexa 594	540/10	570LP	600/25

NOTE: Both Olympus confocal systems share a common multiphoton excitation laser. While each microscope is an independent single photon system, only one can function as a multiphoton system at one time.

Additional/Spare Equipment

The Centre has a collection of additional filters and objectives that can be fitted to the Nikon microscope systems. Additionally there are several spare cameras that can be attached to other microscopes within the institute if needed.

Objectives

Magnification	NA	Contrast Capable	Working Distance (mm)	Series
4	0.13	No	17.10	Plan Fluor
10	0.30	Phase	16.00	Plan Fluor
20	0.45	Phase, DIC	7.40	Plan Fluor
20 (Oil, Glycerol, Water)	0.75	DIC	0.35	Plan Fluor
40	0.60	Phase	3.70	Plan Fluor
40	0.60	Phase, DIC	3.70	Plan Fluor
40 (Water, Dipping)	0.80	DIC	3.50	NIR Apo
60 (Water)	1.20	DIC	0.22	Plan Apo
60 (Water)	1.20	DIC	0.27	Plan Apo
60 (Oil)	1.40	DIC	0.21	Plan Apo (VC)
100 (Oil)	1.40	DIC	0.13	Plan Apo (VC)
100 (Oil)	1.45	DIC	0.13	Plan Apo TIRF

Filters

Excitation	Dichroic	Emission
360/40	400LP	460/50
400/40	565LP	610LP
560/40	595LP	630/60
515/30	545LP	550LP

Cameras

Brand	Model	Colour/Monochrome
Nikon	DMX1200C	Colour (Bayer)
Nikon	DMX1200C	Colour (Bayer)
Nikon	DMX1200C	Colour (Bayer)

Software Analysis and Computer Workstations

- **Standard Analysis** – Used for basic MetaMorph analysis (area stained, wound healing, colony counting, etc.)
    - HP Workstation xw6200
        - Single core Xeon (2.8GHz)
        - 16GB RAM
        - 896MB nVidia 260GTX graphics card
        - 2 x 500GB SATA hard drives
        - Windows XP x64 Service Pack 3
    - Installed Software
        - MetaMorph Premier 64 Bit (Version 7.6.2)
        - Fiji/Image J (Version 1.43d)
        - BioImageXD Beta (Version r1537)
- **High End Analysis** – Used for Imaris and CPU intensive MetaMorph analysis (3D cell segmenting, image filtering, deconvolution, etc.)
    - HP Workstation 8100 Elite
        - 4 Core Intel Core i5 670 (3.6GHz)
        - 16GB RAM
        - 1024MB ATI Radeon HD 6450 graphics card
        - 2 x 1TB hard drives
        - Windows 7 x64
    - Installed Software
        - Imaris x64 (Version 7.0)
        - MetaMorph Premier 64 Bit (Version 7.7.1)

Services Offered

- **Equipment Access** – Access is available 24/7 for all Ludwig staff and students. Staff and students from University of Melbourne Departments of Surgery and Medicine as well as the Walter and Eliza Hall Institute have the same access. Other external users can access the equipment in normal business hours by prior arrangement.
- **Training** – All users, internal and external, are trained to use any equipment prior to use. Training includes the following:
    - A 70 page manual explaining the basics of digital imaging, simple optical theory, fluorescence, widefield imaging techniques, confocal imaging and laser safety
    - One on one tutorial explaining the usage of the system
    - For confocal microscopes 5-10 hours practice is required. This is followed by a competency test
- **Image Capture** – CAM staff can be contracted (on an hourly charge basis) to capture images for internal and external users
- **Experimental Design** – CAM staff can assist users in designing or tweaking protocols to achieve optimal results

- **Image Analysis** – CAM staff can develop image analysis scripts/macros/journals as required
- **Institute Wide Microscopy Support** – Regular maintenance and support is extended to all microscopes and microscopy based equipment within the institute. CAM staff regularly check, clean and service all the microscopes within the Institute.