EQUIPMENT UPDATE

DEPARTMENT OF BIOCHEMISTRY

July 2017

CELL IMAGING

MICROSCOPY

CRYSTALLOGRAPHY

STERILISATION

FRACTIONATION

THERMAL CYCLING

CENTRIFUGATION

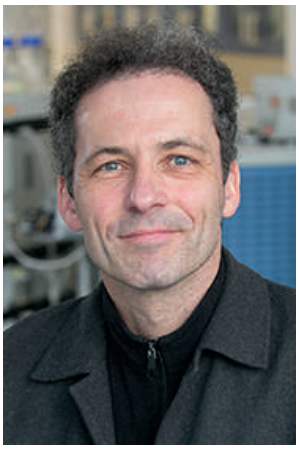
MASS SPECTROMETRY

At last we have the promised "Equipment Special" monthly update for you. It showcases the equipment we have in the Department, both "old workhorses" and some new additions and upgrades with which most of you will not be familiar as yet. Hopefully this collection of information about what's available will spark some ideas in some brains and lead to some good new experimental outcomes!

Some of the equipment has "experts" associated with it who are partly paid by the Department for their services, so do not be shy about asking these people for their help. Other equipment is located in individual laboratories and looked after by volunteers, so be considerate when requesting its use. On that note, the Department has recently purchased an HPLC system (room 1.19) and a colony-picking robot (room 1.16b).

Above all, look after the equipment! It is all expensive to buy and costs a lot to fix, but more importantly, broken equipment means that everyone's experiments get held up, not just yours. Be helpful to new users, but leave it to the experts to do their initial training. Contact Peter Small or Peter Fleury if anything malfunctions - do not try to fix it yourselves!

Experts' contact details:



Torsten Kleffmann: 7867

CPR

Abhishek Kumar: 7542

CPR

Diana Carne: 7542

CPR

Carolyn Porteous: 5190

Centrifuges

Isaiah Cheong: 3570

Microscopes

Robyn Lee: 7872

Thermal cyclers

Sam Jamieson: 7860

Crystallography

Tanis Godwin: 7868

CGL equipment

Lois Martin: 7847

Autoclaves

Andrew Cridge: 4673

PCR/melting assays

Rob Day: 7868

Sequencing

Monica Gerth: 7836

ITC and CD

Nucleotide sequencing

The Cancer Genetics lab has an Illumina MiSeq platform that is available for collaborations looking to develop and test new methodologies by high throughput sequencing. We envisage that successful protocol development will lead to further data gathering in conjunction with the Otago Genomics Facility.

Cancer Genetics has expertise and research materials that may help as a primer for projects looking at single cell and other low input RNAseq experiments, screening CRISPR-Cas9 transformants and highly multiplex amplicon sequencing.

Contact me to discuss how the CTCR MiSeq could help progress your research.

Rob Day

Grace using the AB7900HT qPCR machine

Thermal cycling

The Biochemistry Department has two Roche LightCyclers - an older 480 and a new 480 II. These are currently housed in Lab 316. The older 480 machine is dedicated for use with 96-well plates, and the new machine is used for 384-well plates. These machines can be used for things like qPCR (qRT-PCR, RT-PCR), HRM, and protein melting.

For new users with questions about setting up experiments and using these machines, your first point of call is someone in your lab. Your lab mates are likely to have used the machines before, and have specific expertise relating to your experimental design and protocols that work for your samples. My personal experience is with qPCR and HRM, but for other protocols I can point you in the direction of experienced users in the department, or, for trickier problems, I can help you liaise with the Roche technicians who should know the answer to everything.

If you wish to use either of these machines, you can book in a time slot at https://biochembookings.otago.ac.nz/ . A word of warning - if you use the machines without booking in, you risk your experiment being tossed in the bin by an irate colleague who has booked in. Please be considerate of other users.

If there are any problems at all with either of the machines, please see me immediately so that we can fix it promptly. They are expensive machines that many labs rely on for their experiments, so we want to limit 'down time' as much as possible.

Robyn Lee

The Dearden lab have two Bio-Rad Real-Time PCR (qPCR) Detection Systems the CFX96 Touch™ (96 well) and MJ Mini Mini Opticom (48-well), available for departmental use. These real-time PCR instruments have advanced optical technology (five colours and one FRET channel) and thermal gradient control allowing for either singleplex or multiplex reactions. These machines are designed for both qPCR and High-Resolution Melt (HRM) assays and are compatible with a number of standard reporter dyes (e.g. SYBER, FAM, Texas Red)

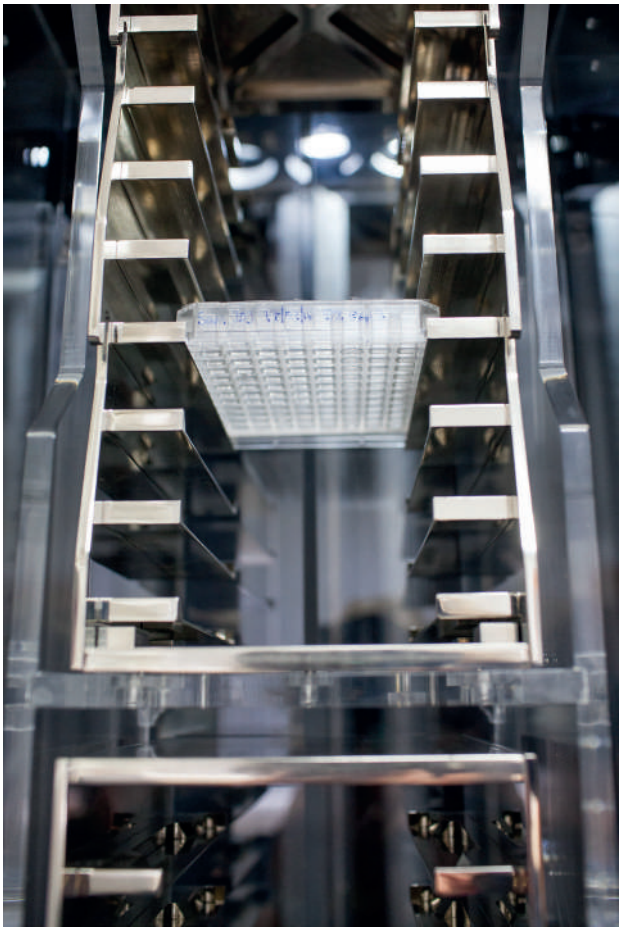
We have experience in qPCR assay development including, RNA extraction, cDNA synthesis, primer design and, selection of appropriate reference genes. Help is also available in qPCR experimental design to determine the total number of experimental and control groups required to ensure that the right type of data, sufficient sample size and power to answer the research questions of interest as clearly and efficiently as possible. We can help with analysis (software available) of qPCR data along with interpretation and presentation of results for publication to meet the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines. MIQE is a set of guidelines that describe the minimum information necessary for evaluating qPCR experiments for publication.

Contact me to discuss how we can help with your research.

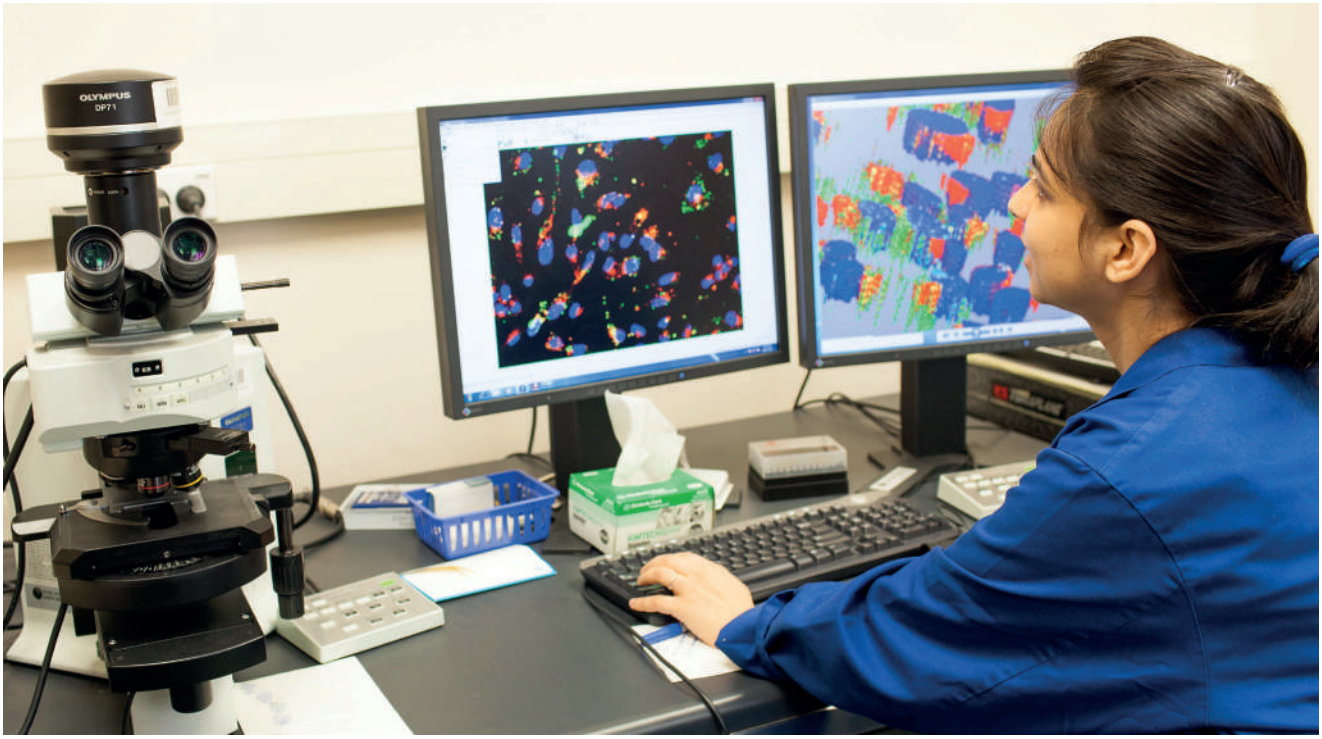
Andrew Cridge

Tanis Godwin is your first point of contact for the following equipment held in the Cancer Genetics lab:

- BioRad ddPCR – AutoDG (droplet generator), plate sealer, deep well PCR block and QX200 droplet reader. QuantSoft software can be installed on multiple PCs.
- ABI 7900HT – qPCR machine for 384 well plates. Can also run TaqMan array cards.
- A 384 well thermal cycler block



Crystal hotel



Analysing fluorescence microscopy images

Cell Imaging

There are two imaging platforms available within the Cancer Genetics lab. The Cytation 5 and Cytell are digital imaging platforms with the ability to image at 4X, 10X (and 20X, Cytation 5 only) a variety of samples (cells, tissue sections etc, but not to be used for bacterial work) in flasks, plates, dishes or on slides. You can perform bright field or flurescence imaging and each platform has an array of analysis tools. The Cytation5 can be temperature controlled and has CO_2 and nitrogen available. I can help with initial protocol set up, and advise on analysis techniques, but many of the proceedures will be very specialist. I can help co-ordinate with others performing similar protocols and can liaise with tech support for additional assitance.

Tanis Godwin

We have a Nikon BioStation system for time-lapse imaging of live cells in room 213. It allows phase contrast and 2-channel fluorescence (FITC, YFP, CFP, DAPI, G-2A filters available) with 20x, 40x or 60x objectives. It is humidified with gas and temperature control, and has ports for injection of reagents during imaging. Low throughput: 35 mm dishes.

See **Liz Ledgerwood** or **Stephanie Hughes** if you would like to use this.

Microscopy

The department's inverted fluorescence microscope was recently upgraded to a Nikon Ti2-A. This new machine has both brightfield and fluorescence capabilities; boasting an expanded filter cube set and a greater range of objectives (4x – 60x). The inverted allows for the imaging of living cells (in flasks with media), along with fixed cells or tissue on slides. The attached camera is monochrome, which means that captured fluorescent images are false-coloured in the imaging program and only single colour stains should be visualised in brightfield (multiple colours cannot be differentiated in greyscale images).

The upright fluorescence microscope is an Olympus BX51, and has a more limited range of objectives and filters but is perfect for imaging of fixed cells or tissue on slides. It also comes with a more advanced version of the same imaging and analysis software as well as a monochrome camera.

I provide the compulsory basic training on the microscopes before users get started and provide help specific to their experimental design. Users will need to request and book ahead due to my own personal lab work schedule (which on occasion takes me out of the department). A quick cheat sheet on how to get started is up by the microscope but any further incidental assistance can be requested.

Both the inverted and upright microscopes are available for trained users to use in room 213, and you can find me in the NDD Lab (room 216/217).

Isaiah Cheong

The Cancer Genetics lab has an inverted microscope with colour camera, it is for bright field work only (capturing H&E, IHC images etc). See Tanis if you want access, she will direct you to Isaiah for help if needed.

Tissue culture stocks:

Cancer Genetics have a substantial stock of cell lines available. Please ask **Tanis** for specific cell lines rather than by cell type.

Protein analysis

Mass Spectrometry at the Centre for Protein Research

We operate three outstanding state of the art mass spectrometers that are designed and set up to tackle a wide range of your research questions.

Are you interested in:

- identifying or characterising your protein and its modifications?
- finding less than a femtomole of your molecule in the complexity of the entire proteome
- analysing a full proteome?
- quantifying and comparing full proteomes?
- ... etc

Or are you more interested in detecting smaller molecules such as

- metabolites
- lipids
- other small analytes
- ...etc

Then come and visit us in our brand new lab area, Rm 111 in the centre of the first floor, to discuss your project with us. We will try to tailor the workflows for the best outcome of your experiments.

Maybe you are interested in learning how to operate these enigmatic mass spectrometers by yourself and how to analyse the data through our comprehensive software pipelines. We provide extensive training and strongly encourage "self service".

Do you need more details? You can visit our website (http://cpr.otago.ac.nz/) or BETTER come to the CPR and talk to **Diana**, **Abhishek**, **Mike** or **Torsten**.

Isothermal calorimetry

This is widely viewed as the 'gold-standard' for determining binding affinities of protein-ligand interactions (best suited to interactions with moderate to high affinities (10 nM to 100 μM Kd).

Our instrument requires relatively large amounts of protein (sample chamber is ~1.8mL) and is low-to-medium throughput, however, with careful experimental set-up, can provide a wealth of thermodynamic data.

Inside the new CPR headquarters

Circular dichroism

The primary use is in analysing the secondary structure of macromolecules, particularly proteins - including how the secondary structure changes in response to the environment, e.g. temperature or pH.

Contact me for training before using these devices.

Monica Gerth

SPR and Blitz

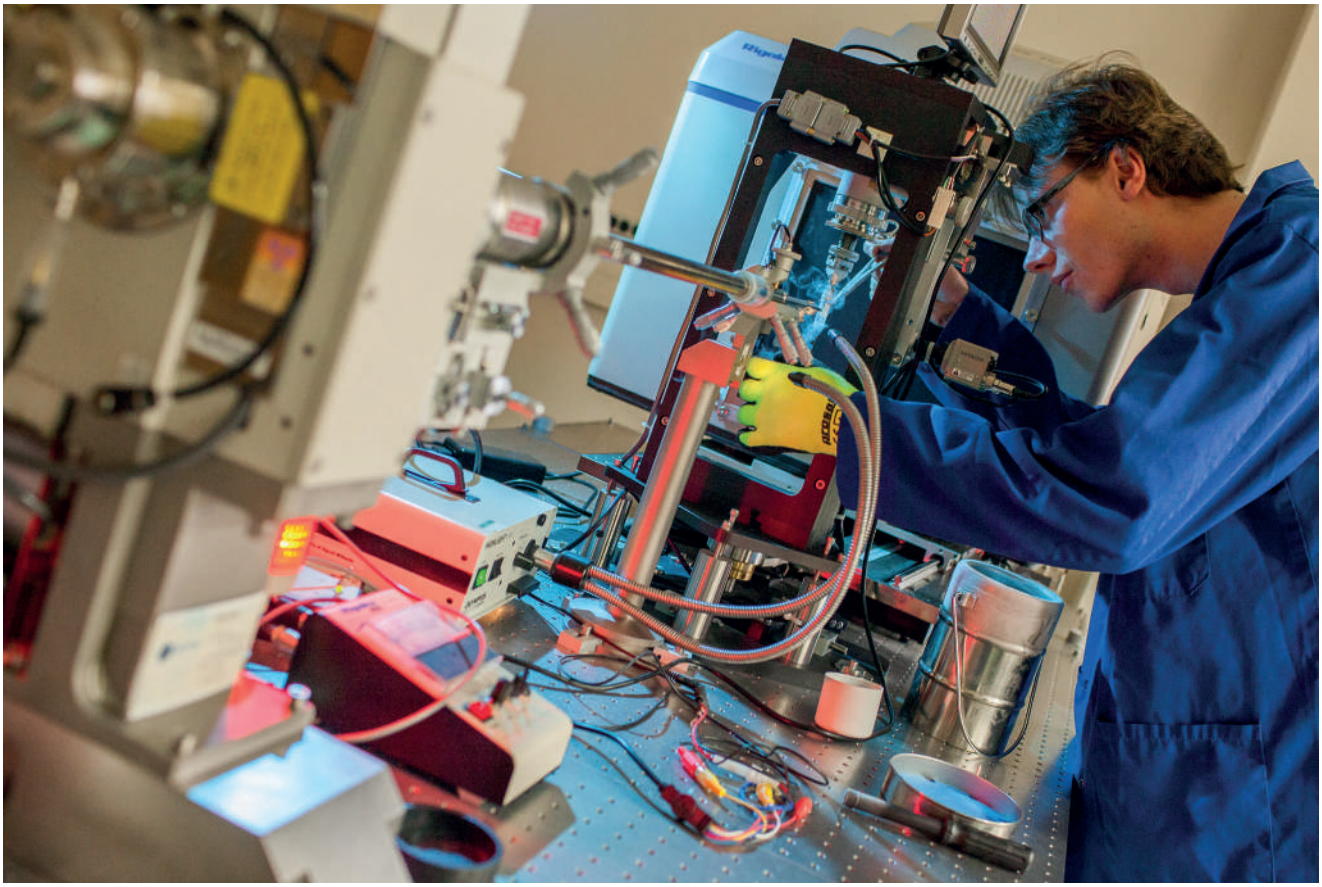
The BiaCore and Blitz are two different technologies for investigating protein-protein or protein-ligand interactions, where one component is immobilised on a surface. They can be used to calculate binding affinity (analogously to solution methods such as ITC), but also provide additional information about binding kinetics. They require much smaller amounts of material than ITC, but require more optimisation to identify suitable buffers and surface immobilisation chemistry.

See me for training before using this equipment.

Peter Mace

Emulsiflex C3 cell homogeniser.

This is a new addition to the department as of July 2017. It can be used to lyse many types of cells (*E. coli*, mammalian, mycobacteria) when the volume is over 20 mL. See **Helen Opel-Reading** or **Kurt Krause** to arrange instructiuon on its use.



Pavel with the X-ray source

Crystallography

The mosquito is mostly used for setting up crystal plates although it is also useful for pipetting small volumes (100nL up to 1.2µL) into 96 or 384 well plates. The software is quite straightforward and generally adaptable to suit your needs. I am available by appointment to take new users or current users through setting up a program and/ or optimising an existing program. I am also usually available, if experiments allow it, to try and rectify any issues

The crystal hotel makes monitoring your crystallography experiments incredibly convenient. With around an hour introduction/ login experiments can be monitored from any university computer. It has many powerful tools to aid you in determining whether you have protein crystals or not. When I take you through using the mosquito I will also take you through the hotel and software associated with it.

Sam Jamieson

Once you have crystals, expertise in collection and interpretation of crystallographic data resides within research groups. If you want to solve the three-dimensional structure of your favourite protein, nucleic acid or macromolecular complex, but your group does have the expertise, consider collaboration with one of the groups in the department which does. If you have recently hired an experienced crystallographer to bring that expertise into your own group, please talk to Sigurd Wilbanks about independent use of the diffraction equipment. There are training requirements and an annual charge for each group. To receive notices about the crystallography suite and upcoming trips to the Australian Synchrotron, join the CrystalClub email list - Adam Middleton (adam.middletone@otago.ac.nz) can sign you up.

Sigurd Wilbanks

Autoclaves

Purpose

Reliable sterilisation with moist heat requires temperatures above that of boiling water and this can be achieved by steam under pressure in an autoclave.

Autoclaving is considered the most dependable method for sterilising media & laboratory equipment,unless the material/equipment to be sterilised can be damaged by heat or moisture. It is also used for decontaminating biohazardous waste.

Autoclaves do not remove chemical contamination.

Sterility monitoring

It is good practice to use a chemical indicator (e.g., autoclave tape) with each load placed in the autoclave – this will alert us to any problem there may be.

Autoclave sterility monitoring is carried out every 14 days using Biological Indicators which contain *Bacillus stearothermophilus* spores, a microorganism that is inactivated when exposed to 121°C saturated steam for a minimum of 20 minutes.

Mercer Medical calibrate & validate our autoclaves annually.

Use of autoclaves

Before you use an autoclave for the first time see your lab supervisor who will show you how to use it – there is a training record with each autoclave that you will need to fill out.

Lois Martin (Lab229) is able to take you through the basics if your supervisor/experienced lab member is not available to do this.

The standard operating procedures can be found on the wall beside each autoclave. This tells you what type of load & quantity (volume in mL or number of bags) can be put into each run.

Record Keeping

An autoclave log needs to be filled in with date, time, and operator's name, contact information (Laboratory, room number, phone number) and type of material sterilised/cycle used.

Lois Martin

Ultracentrifugation

There are two ultracentrifuges available to the department. Both are housed in the instrument room on the 2nd floor (room 215).

The Optima XE-90 is the larger of the two and is used for volumes greater than 4 ml, while the Optima MAX-TL is the table top ultracentrifuge which is for samples of 1 to 4 ml.

The MAX-TL has two different rotors which can be used, the smaller of the two (rotor with the red lid) holds 1ml tubes and the other rotor (yellow lid) holds tubes that hold a volume of 4ml. FYI these rotors are often stored in the 2nd floor cold room.

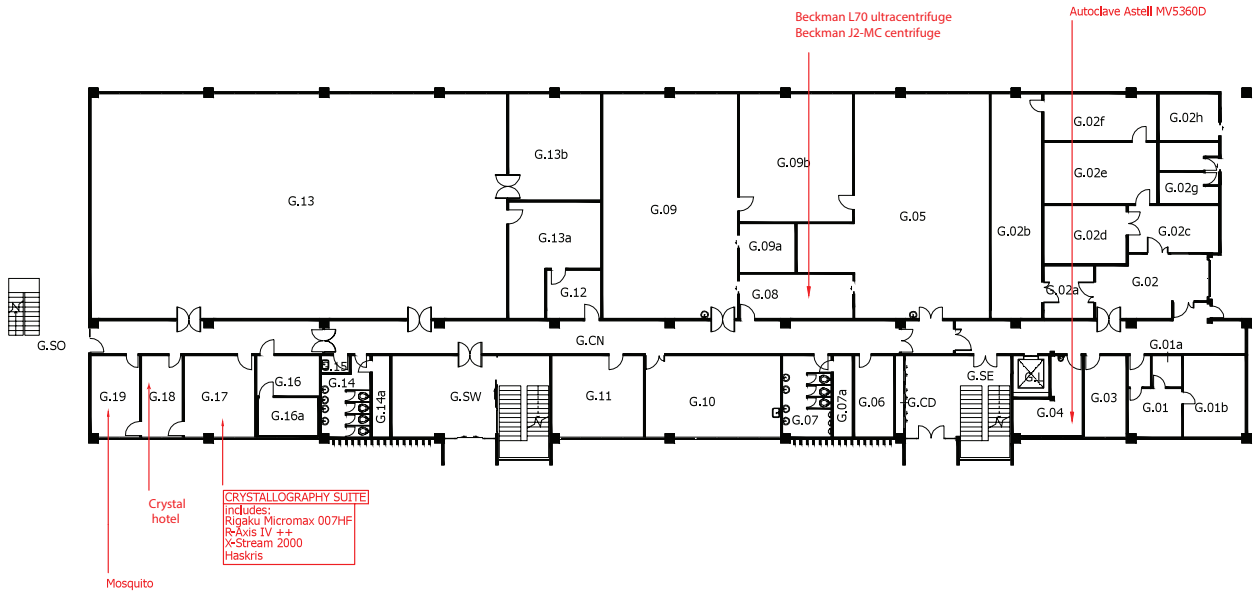
I have found the biggest obstacle so far with people on planning to use the ultracentrifuges is the lack of planning. Due to the expense of ultracentrifuge tubes we no longer hold any stock of these tubes so people have to be aware that before they are able to use either of the centrifuges they will have to investigate which tubes they will need and then place an order. Sometimes it can take a while for the tubes to arrive. Some labs do have a supply of tubes, but only the ones they would use for their purposes and they may not have excess of these to lend. There is a vast array of choices when it comes to tubes but they all have different purposes, so investigate thoroughly beforehand.

Please see me if you need help with the centrifuges.

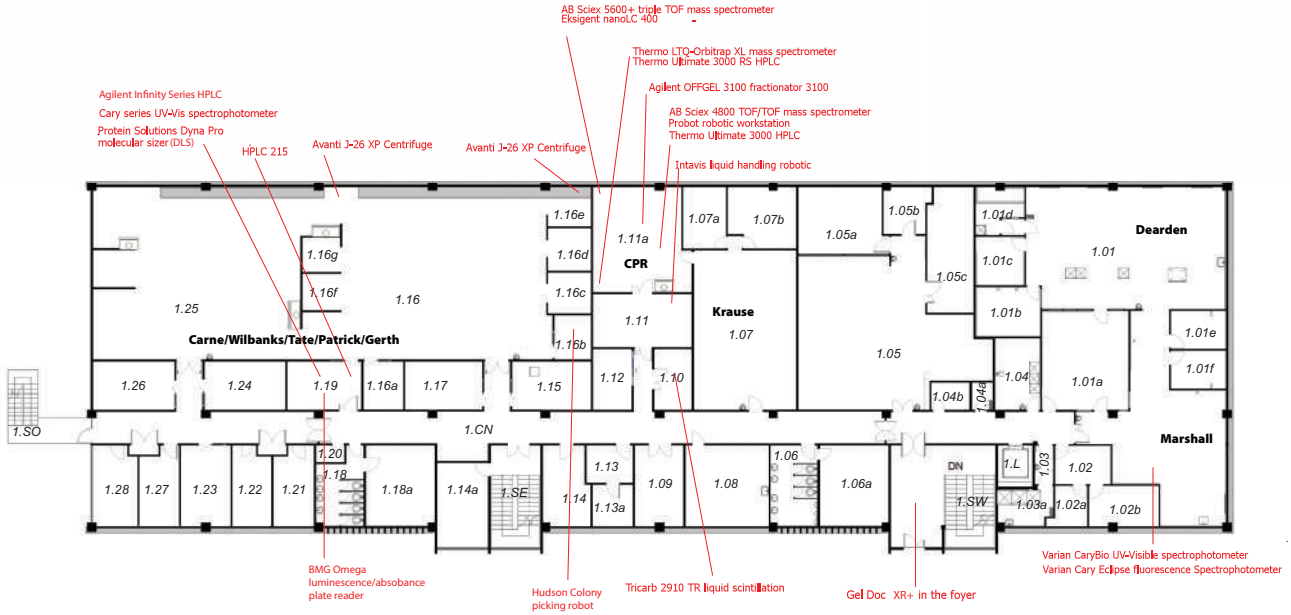
Carolyn Porteous

WHERE IS EVERYTHING?
WHAT IS THAT MACHINE?

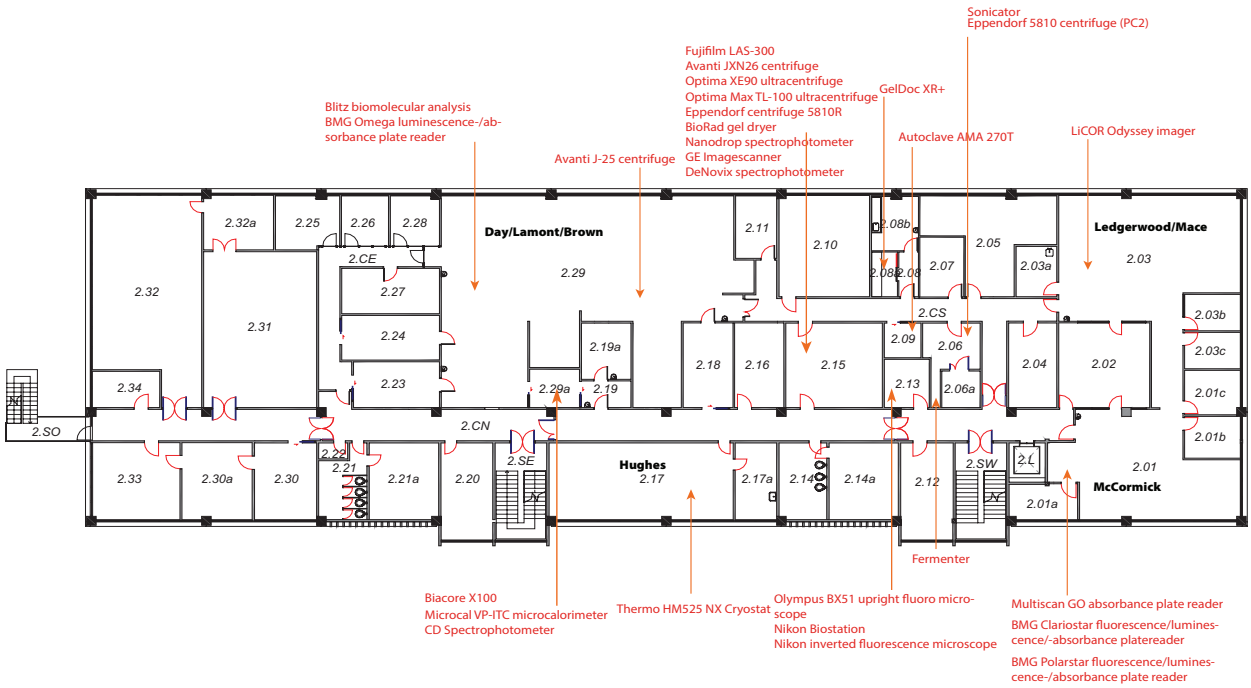
Ground floor



First floor



Second floor



Third floor