NDMRB Flow Cytometry Suite
NDMRB-SOP-022

Background
The NDM RB flow suite comprises an AutoMACS, two Cyans, one Fortessa and one Cat 3-abled MoFlo machines. The AutoMACS is situated in the Kennedy and the Mo-Flo is situated at the WIMM.

NDMRB occupants and OCDEM have preferred user status (same costs as all users from ORC network). All other users from Oxford University will be considered, but costs will be higher, note that depending on the type of grant used, VAT may need to be charged. Users external to the University of Oxford will pay a higher cost and VAT will be included.
Please enquire with Tiph Bouriez-Jones to find the updated pricing.

Personnel related to Flow Suite
- Facility Manager – Drew Worth (DW) Andrew.worth@ndm.ox.ac.uk (6)17600
- Lab manager for ordering – Tiphaine Bouriez-Jones (TBJ) tiphaine.bouriez@ndm.ox.ac.uk (6)12918

Training and Access
1. NDMRB Building induction with Ross Macrae ross.macrae@ndm.ox.ac.uk – held weekly on Thursday at 10am. Contact NDMRB reception to sign up.
2. Lab induction with Tiph Bouriez-Jones tiphaine.bouriez@ndm.ox.ac.uk - held weekly on Monday at 11am. Contact TBJ to sign up.
3. CyAn or Fortessa training with Drew Worth andrew.worth@ndm.ox.ac.uk – arrange on demand, contact Drew to book a session.
4. Validation of training by Drew Worth as the user runs his/her first experimental samples – this will allow access to the online booking calendar.
5. Access form to be signed by Seph Borrow or Tiph Bouriez-Jones once Drew has validated training.

Risk assessment
Before any work can start an appropriate risk assessment must have been carried out, taking in account the potential hazard of the cells, microorganisms, vectors, stains and chemical that may be present in the samples. It is likely that a single risk assessment will cover a complete experiment or group of experiment. However, it is essential to reconsider biosafety issues if any aspects of the procedure changes. See Biosafety Risk Assessment – appendix 1.

Note that risk associated with processing cells through the cells analysers (Fortessa and Cyan) are different to risk associated with cell sorter (MoFlo) since the former is a closed system and bio-containment is possible whereas cell sorters produce aerosols and containment is difficult and complicated.

Risks should always be minimized therefore cells should be fixed prior to analysis. However, if fixation is shown to have an adverse effect on data quality, it is possible to analyse fresh cells subject to an appropriate risk assessment and following specified precautions.

The majority of sorts carried out require the cells to be unfixed. Mouse cells are usually pathogen free and associated risk are low. Cells from non-human primates and from unscreened human samples are considered high risk and should only be handled on a sorter utilising appropriate bio-containment measures (normally CL3 or equivalent). Screened human samples may be handled at CL2 level with additional aerosol containment precautions.

CL2 Laboratory rules

The Flow Cytometry operates in a CL2 laboratory and local rules must be followed

- Eating, drinking and smoking are absolutely forbidden in the laboratory.
- Lone working is permitted if a suitable risk assessment has been carried out.
- The laboratory door must be kept closed at all times.
- Personal Protective Equipment (PPE) must be worn whilst using Flow cytometer:
  - Blue labcoat
  - Nitrile gloves
  - Safety spectacles
  - Closed shoes

Gloves should be removed before leaving the laboratory or when known to be contaminated. Laboratory coats should be changed every week. Open sandals must not be worn in the laboratory.

Clean Surfaces

The following surfaces in the FACS Flow suite should be considered clean and **must not** be touched with gloved hands:

- Door handles and lock
- Light switches

Status of keyboards and control pads are stated.

Waste procedure

- Liquid waste
  - Must be treated with Virkon tablet for at least 30min and then poured down the sink with copious amount of water.
  - Strain out any solid waste and place solid waste in autoclave bag.
- Black Sacks (black)
  - For domestic, non-contaminated waste only. Cardboard may be recycled and kept separately (blue bins).
- Autoclave bags (silver)
  - All contaminated waste, including all gloves (whether contaminated or not). Please do not over fill autoclave bags, when full tie with a cable tie and put into the relevant collection bin.
  - Stripettes should be disposed of in a manner that does not puncture the autoclave bag (round cylinder bins to be used to bag up pipettes before being placed in grey autoclave bins.
- Sharps bins
  - For disposal of needles and small glass items only. When full inform the laboratory manager and they will dispose accordingly.
  - Disposal of syringes and multi-step dispensers.
- Glass bins
  - Clean broken glassware – clean is defined as had no contact with chemicals or biological substances. Clean broken glassware can be disposed of by collecting the broken item and disposal straight into the lab glassware bins.
  - Chemically contaminated broken glassware: Glassware which has come into contact with toxic substances must be collected in a clearly identified bin and collected by the HSO.
  - Biologically contaminated broken glassware: Glass ware that has come into contact with biological substances must be collected and put into a dispo jar. This

glassware must be autoclaved before it can be disposed of using the normal channels.

All Blood products MUST BE DISCARDED AS CLINICAL WASTE.

Upkeep of machines

This will be provided by Drew for all three machines. It is estimated that this will take 2 hours maximum per week, and comprises weekly check, systems clean and running of QC beads. Service contracts are managed and held by Leo Dempsey, with advice from the FACS committee.

Consumables and reagents

Central FACS machine-related reagents and consumables will be FACS flow fluids, FACS QC beads, disinfectant, paper towels, and gloves. These will be ordered as part of the stores for the first floor labs and managed by TBJ. AW will check (mid-week) and TBJ (every Monday) that the stocks are sufficient for the week. TBJ will order as required. Funds for this will be drawn from the fee per hour paid by each user.

Tissue Culture

Flow users do not get access to the Tissue Culture suite though the two rooms are connected. Users are asked to avoid disrupting air flow (i.e. repetitive opening of the door) not to disrupt sterile work being carried out in the safety hood. Tissue Culture consumables are only available to Tissue Culture users and will be re-charged accordingly.

CyAn ADP Detector Block and Optical Filter Layout

Blue excited detectors

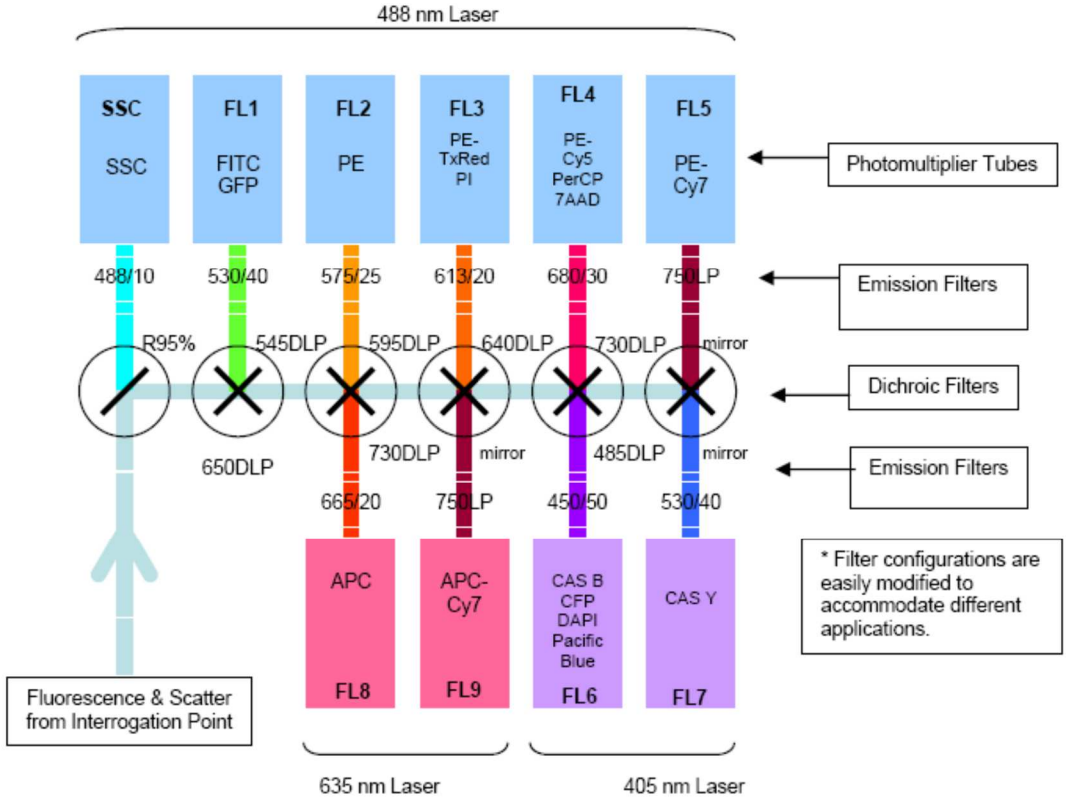
FL1	510 to 550 nm	FITC, GFP
FL2	562.5 to 587.5 nm	PE
FL3	603 to 623 nm	PE-TexasRed, PI
FL4	665 to 695 nm	PE-Cy5, PerCP, CyChrome, 7AAD
FL5	>750 nm	PE-Cy7

Violet excited detectors

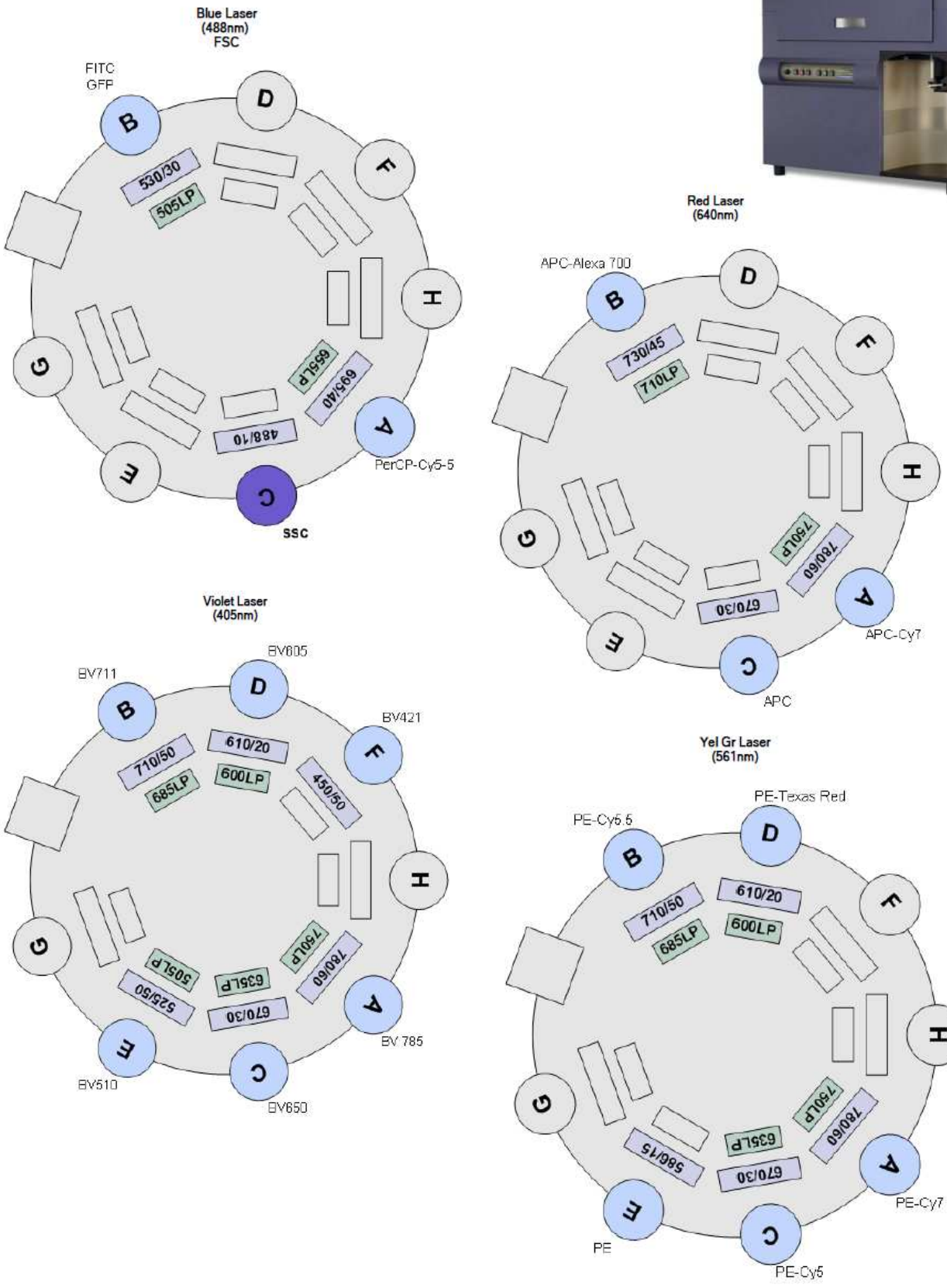
FL6	425 to 475 nm	Pacific Blue, Cascade Blue, DAPI, CFP
FL7	510 to 550 nm	Pacific Orange, Cascade Yellow

Red excited detectors

FL8	655 to 675 nm	APC, Cy5
FL9	>750 nm	APC-Cy7, APC-Alexa750



Fortessa Detector Block and Optical Filter Layout



Fortessa Detector Block and Optical Filter Layout

Brief description:

The AutoMACS Pro Separator by Miltenyi is an automated high-speed immunomagnetic cell separation system with the ability to process multiple samples at a time.

Many cell types from several species and tissue types, including whole blood, can be processed with the AutoMACS Pro with the use of immunomagnetic beads.

Cells can be separated using positive selection, depletion or untouched isolation programs. It can sort more than 10 million cells per second from a samples of up to 4×10^9 total cells.

The instrument has a touch screen and easily navigable menus.

The system reduces manual handling of samples and increases reproducibility.

Microbeads do not require to be removed, cells can be sorted or analysed on Flow Cytometer directly after staining.

Cost:
Currently no cost is associated with the use of the machine, users will need to bring their own buffer, beads and tubes.

Location:
Kennedy Institute, Old Road Campus
The unit is in a dead-air hood, in the FACS room on the first floor.

Main contact:
Jonathan Webber jonathan.webber@kennedy.ox.ac.uk
Training will take less than 15 minutes. Booking can be made once the user has been trained

For more information on the AutoMACS system, please visit Miltenyi.com

Biosafety Questionnaire:

Date submitted:_____
Group: _____
Principal investigator: _____
Investigator name:_____
Email: _____
Phone: _____

Brief description of project

List type and source of cells

☐Mouse ☐Human ☐Other
(i.e. PBMC, thymocytes, cell lines…)

Is the sample known or likely to contain pathogens?
Refer to ACDP "the approved list of biological agents"
List agents and include hazard group

Has the infectious agent been inactivated?

☐Yes ☐No ☐not applicable
If yes, provide details of the method and validation

If the samples are of human origin, are they screened for known pathogens?

☐Yes ☐No ☐not applicable

If yes, confirm that only pathogen free samples will be used. If no, state why screening was not done.

Were the cells transformed using a virus such as EBV, HTLV-1, herpes saimiri?

☐Yes ☐No

If yes, list details:

Were the cells genetically engineered?

☐Yes ☐No

If yes, provide a brief description, list the risk assessment number and attach the document

Was a gene therapy virus (adenovirus, retrovirus, lentivirus, herpvirus…) used with the cells?

☐Yes ☐No

If yes, describe the method, attach the vector map and show packaging cell line

Have the cells been tested for mycoplasma infection?

☐Yes ☐No

If yes, give test date and results. Test must be performed just prior to use in the FACS lab.

Will the samples be fixed prior to use in the FACS facility?

☐Yes ☐No

If yes, provide details of the method. If no, state the reason why.

If sorting, will the collection media contain any hazardous substances?

☐Yes ☐No

If yes, provide details and attached COSHH assessment.

If sorting, will the samples contain any other potentially hazardous substances?

☐Yes ☐No

If yes, proved details and attached COSHH assessment

If samples contain pathogens, please provide details of a suitable disinfectant and validation of effectiveness.

Declaration

Name (PRINT): _____

I have read the questions above and certify the information provided is correct

Signature:	*Date:*

To be completed by FACS facility manager or nominated deputy

Risk assessment approved ☐Yes ☐No

Approved by: _____

Date approved: _____

Risk assessment Number: _____

Comments

NDM Research Building
Staff Training form
First Floor FACS suite

Name:

Group:

Status:_____
Grant number: _____
Contract start date:_____
Contract end date:_____

All persons working in the NDM RB Flow suite must have undertaken the following inductions and have provided a suitable RA for their work:

1. NDM RB Building Induction (Ross Macrae)
2. NDM RB FACS Suite Induction (Tiph Bouriez-Jones)
3. NDM RB FACS Equipment induction and training (Andrew Worth)

The rules set out within Standard Operating Procedures and Risk Assessment must be read, understood and followed by all persons working within the NDM RB. Personnel have a duty of care to themselves and to those around them.

Signature of Trainee: Date	
Signature of Tiph Bouriez-Jones: Date	
Signature of Andrew Worth: Date	

Please hand this form together with your access form to either Persephone Borrow or Tiphaine Bouriez-Jones for them to countersign the access form to the FACS Flow suite.

This form together with the access form should be handed back to reception for card access to be activated.