**Old Road Campus FACS Facilities Audit and Future Requirements**

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**Summary**

World-class FACS (cell sorting and cytometry) is essential for internationally competitive biomedical science. It is especially crucial in immunology, but also is increasingly used in studies of tumour heterogeneity, and in genetic and small molecule screening. Although Oxford has an excellent FACS facility based at the Weatherall Institute for Molecular Medicine (WIMM), the Old Road Campus (ORC) currently has limited capacity, and lack of investment and aging equipment has reduced international competitiveness. With transfer of groups with strong interests in immunology from the WIMM to the ORC, the construction of the Target Discovery Institute (TDI) building, and the arrival of the Kennedy Institute of Rheumatology (KIR), there is an increasing and urgent requirement for world-class FACS facilities on the ORC.

 A review of current facilities has been undertaken, and open discussions were conducted with as many major users as possible, including The Jenner Institute, Oxford Center for Diabetes, Endocrinology and Metabolism (OCDEM), The KIR, Oncology, groups migrating to the ORC from the WIMM, users located in the Centre for Cellular and Molecular Physiology (CCMP) and the Wellcome Trust Centre for Human Genetics (WTCHG) and others. These discussions have led to the principle recommendation that a world-class FACS facility be established on campus, ideally in the new TDI building should space be available, and effectively coordinated with the FACS facilities within the Kennedy Institute by establishing a campus-wide user group. Prof. Mike Dustin, who will join the KIR in September, would be willing to chair such a user group and has experience with chairing a similar user group that coordinated facilities across NYU Medical Center.

Details of the facilities audit and recommendations in detail are outlined below.

**Current Equipment and Infrastructure on the ORC**

An inventory of FACS and flow cytometry for the ORC is provided in Appendix II. The Kennedy Institute’s CytoF Mass cytometer is located in the Botnar Institute, about 10 minutes walk from the ORC.

Currently two cell sorting services are provided on the ORC, and a third will be established when the KIR re-locates from London:

The Jenner Institute

Andrew (Drew) Worth runs a cytometry and sorting service for the Jenner Institute located in the Green Building. Both services are available for Jenner and non-Jenner users. Drew also provides Jenner’s IT support.

Jenner currently houses a DakoCytomation (Beckman Coulter) MoFlo with a 488nm coherent Sapphire 200mW adjustable laser and a 633nm HeNe laser with 75mW. They can perform a 4 way sort into tubes or sort into 96 or 384 well plates.

The MoFlo sorter was purchased in 2005 and will cease to be supported by the manufacturer next year (2014). Although a laser upgrade is possible at an estimated cost of £23k the analogue electronics in this machine will become increasingly difficult to source. Beckman Coulter do offer an upgrade route to MoFlo XDP specification at a cost of £110k to include replacing current red (635nm) laser with 100mW red + new detectors + new PC + new electronics. Addition of an additional laser would raise costs to £150,000 and new health and safety requirements indicate that cell sorting of lentiviral-infected cells may need a laminar flow hood associated with the sorter. In total an upgrade would costs in the order of £170,000

The sorting service is currently used to 25% capacity and service contract is currently £6000 pa excluding the main laser.

Jenner also houses a DakoCytomation (Beckman Coulter) CyAn cytometer (3 laser, 9 colour) purchased in 2005 that is used to 20% capacity as well as a BD LSRII cytometer (3 laser, 15 colour) purchased in 2007 that is used to 60% capacity. However, the current configurations lack a Yellow/Green laser that is now being widely used by competitor labs and a new laser cannot be added to the current equipment.

Service provision for Jenner users is currently £70 ph and for non-Jenner £100ph with assistance (£80 without) with a discounted rate of £54 ph with assistance (£27 without) for the WTCHG.

Current usage of the Jenner facility indicates 70% usage by Jenner, 25% WTCHG. and 5% other.

Although set up as a Small Research Facility, the Jenner cytometry facility has seen no investment for the past 5 years, the laser options are restricted, not upgradable, and are now lagging what is standard elsewhere, leading to loss of competitiveness.

Oncology /The Gray Institute

The Gray Institute located in the Green Building has a flow cytometry and sorting service run by Mick Woodcock.

The sorter is a 20-year-old FACS Vantage that currently has only one operable laser. Restoring full function to this equipment would cost in the region of £25,000 but the equipment is considered largely obsolete, with a sorting capacity of 3000 events per second, compared with more modern sorters with capabilities up to 70,000 events per second. The Current equipment is also much less user-friendly than more modern FACS machines.

The Gray also contains a FACS Calibur cytometer that is several years old but can provide basic cytometry. A service is provided to members of Oncology but is currently unavailable to non-oncology users.

The facility is currently underused as a result of a combination of lack of awareness and the slow sort speed that requires a full-time operator for several hours to get a single sort done well.

The Kennedy Institute of Rheumatology

The KIR will move to the ORC in 2013 and has a major interest in both cell sorting and flow cytometry. Their resources will include a number of flow cytometers including a new BD Fortessa and an upgraded BD LSRII, as well as a new FACS Aria III cell sorter. Although the cell sorting and flow cytometry capacity within the KIR will be available to other users on the ORC, its planned expansion means that there will be limited capacity to share with non-KIR users.

The Flow and CytoF facility will be run as an integrated unit by an expert operator, Parisa Amjadi, who will recruit and train an assistant. There will be a user group supervising this activity, chaired by Prof Michael Dustin who also has a strong interest in coupling flow cytometry to high-throughput screening.

In addition, the KIR has recently obtained a CytoF mass cytometer that uses antibodies coupled to heavy metals to determine gene expression. Currently up-to 50 different antibodies can be used simultaneously. This is a major technological advance and only two CytoF machines are currently in the UK. The KIR CytoF is being housed in the Botnar Institute 10 minutes walk from the ORC and is undergoing trials. This instrument will be available for non-KIR users, and other labs on the ORC with interests in immunology and tumour heterogeneity would be keen to use this technology.

Additional flow cytometry facilities on the ORC

Basic flow cytometers are found in several locations including the Ludwig Institute and Oncology/Clin Pharm (LaThangue Lab) in the Green Building, and the WTCHG (Cornall lab).

The McMichael/Rowland-Jones/Borrow groups will bring a Beckman Coulter flow cytometer with them when they move to the TDI in Summer 2013 but will not import a cell sorter that is essential for their work.

Off-Campus Facilities

A world-class cell-sorting service facility is found at the WIMM, and equipment for cytometry and sorting are also found in the John Radcliffe Hospital and a facility is also accessible at the Dunn School. These sorting facilities can be accessed for a fee, but would not be ideal for groups based on the ORC.

**Sorting and Cytometry needs**

The major current uses for both cytometry and sorting are for immunological applications with the principle users being, in addition to the KIR, the Jenner Institute, the McMichael/Rowland-Jones/Borrow groups and the Translational Gastroenterology Unit (TGU) that will translocate from the WIMM to the TDI building, OCDEM, and the Channon and Cornall labs (WTHGU/CCMP). Some groups from OCDEM have been using the cell sorting service housed at the Dunn School since access is available at short notice.

Potential future users for sorting will include those with an interest in tumour heterogeneity from the Ludwig Institute (Goding and Lu Labs), Oncology (Eric O’Neill, Seymour, La Thangue Labs) and those interested in combining cell sorting with deep sequencing in Oncology (Middleton, Cazier)

In addition there is likely to be an increasing requirement for high-throughput cell sorting/cytometry coupled to drug, shRNA or gene expression screens as a part of the target discovery program in the TDI (Daniel Ebner), Michael Dustin of the KIR, and possibly other users in the TDI (eg Stefan Knapp, the British Heart Foundation Centre).

**Current Limitations**

Current limitations to flow cytometry and cell sorting on campus are reducing competitiveness and are acting as a barrier to high-quality world-class science.

Limitations to use of the Jenner cytometry and sorting service include:

• Restricted laser options and aging equipment that are reducing competitiveness

• Lack of ‘out of hours’ availability and easy access

• Limited trained personnel, with no back-up

• Lack of rapid availability; those with clinical samples to process may only have very short notice of tissue availability.

• High cost is a barrier to using the facility for some potential users

• Perception that the major facility on campus is a ‘Jenner facility’ rather than a campus facility.

**Recommendations**

The principle recommendation is that a world-class core facility be established on the ORC that would provide sorting and cytometry services campus-wide.

The establishment of an ORC FACS facility has strong support from all major users including Jenner, all groups migrating from the WIMM, Oncology/Gray, OCDEM, the KIR and others. Importantly this needs to be done soon to avoid groups arriving from the WIMM not having immediate local access to their primary research tool.

Since maintenance and use of FACS equipment requires highly trained and dedicated staff, the concentration of high-end FACS equipment in a new facility has the key advantage that multiple machines can be maintained and managed by a limited number of staff. This would effectively reduce running costs, increase usage per machine, reduce redundancy, and facilitate the provision of back-up of associated staff. Should FACS facilities be fragmented across campus, it is likely that each machine would be relatively underused, and require staff to run each separate piece of equipment with little ability to provide back-up. The establishment of a FACS facility would therefore be more efficient in terms of purchasing power, running costs and manpower.

Current cytometers, such as those associated with specific groups (Ludwig, Cornall and LaThangue labs and the LSR-II in the Jenner), would remain in their current locations given their convenience for their associated users. However, as these resources become obsolete, user-friendly low end-cytometers housed in a core facility would act as an effective replacement.

Location

A new FACS facility housing the anticipated equipment needed would require approximately 30-40 m2. Three locations were initially considered: The Jenner Institute; The KIR; or the new TDI building. For reasons set out below, the TDI building is the preferred choice should space be available, and it may be easier to obtain funding for a new cross-departmental campus-wide facility than for upgrading an existing service. However, part of the Jenner may shortly move to another location on the ORC, and they have indicated that they may wish to reconsider the best location for any FACS facility if the move happens.

At present the Jenner Institute is short of space and is not able to accommodate any additional equipment/services, though the Jenner does have a highly-trained facility manager.

The KIR has yet to arrive on Campus, and has limited space reserved for its FACS facility in its new building on campus. Marc Feldmann, Director of the KIR, felt that the campus would be best served by establishing a new facility within the TDI building (or other location) and providing a high degree of coordination, cooperation and backup between the KIR FACS facility and any new facility.

The TDI also requires a cell sorter that ideally would be positioned close to the infrastructure associated with the drug discovery program and the screening facilities, especially since there may be health and safety issues with transporting virus infected cells in microtitre plates across campus.

The use of flow cytometry as a readout for screens is also likely to increase, especially with the arrival on campus in September 2013 of Prof Mike Dustin of the KIR.

Management

Should a new facility be established, the management and funding structure of the current facility in the Jenner would need to be reconfigured to accommodate new users and their different funding circumstances and user demands.

One possibility is that the new facility be managed by a committee of users similar to that which currently runs the Jenner facility, that would include representatives from all major current and potential users (eg Jenner, Andrew McMichael, TGU, OCDEM, KIR, Oncology, BHF) who would provide input for future upgrades to the service as well as oversight of charges etc. A campus-wide committee/user group should be established to coordinate any new facility with that in the KIR. Prof Mike Dustin from the KIR, who is keen to see and coordination of FACS services across campus, would be willing to chair such a user group. He has experience with chairing a similar user group that coordinated facilities across NYU Medical Center.

Operation

The operation of FACS equipment, especially cell sorters, requires dedicated and highly trained personnel. If a new facility were to be established on the ORC, with the inclusion of Jenner’s MoFlo cell sorter and Cyan cytometer, then a recommendation would be that Drew Worth, the current manager of the Jenner facility, be in overall charge of the equipment since he has the necessary in-depth expertise. However, a second FACS service operator would be needed for any new facility given the increased capacity, because Drew would continue to spend time in the Jenner helping analyse data generated, and as back-up to cover holidays/sickness, or to manage the service should Drew depart. His second role as Jenner’s IT manager, would need to be taken on by someone else. It is possible that the current service provider in Oncology, Mick Woodcock could take on the role as the second FACS facility operator, although whether he would be the ideal choice remains an open question. The TGU also has a trained operator associated with it.

Coordination with the KIR, especially their trained operator Parisa Amjadi and her assistant that is currently being recruited, may enable a further level of cross-campus operator back-up/service provision to be achieved.

To facilitate out-of-hours use, especially by OCDEM, it is recommended that a limited number of registered users be trained in use of the facilities, in addition to core service managers. And in addition to high-end machines that need a highly trained operator the facility would require a low-end machine that is highly user friendly.

A computerized booking system needs to be established to enable machine time to be reserved online that would also facilitate monitoring of usage.

Equipment

The current equipment that could be transferred to any new facility would include the Jenner’s MoFlo sorter and Beckman Coulter Cyan cytometer, and the McMichael group’s Beckman Coulter Cyan cytometer.

The MoFlo is flexible, but needs a dedicated operator, and as mentioned before needs to be upgraded to include a hood and additional lasers to restore support from the manufacturer and extend its lifespan. The cost for this major upgrade would be in the order of £170k

To maintain international competitiveness, and to have the capacity necessary for cross campus usage, the facility would also need to include:

• A 4 laser sorter including a yellow/red laser (such as 561nm), since there is only one sorter with a similar laser in Oxford. A FACS Aria is recommended as it is relatively user-friendly (approximate cost up to £370k ). A technology to watch if sort capacity is an issue is the instrument from i-Cyt/Sony in which two high-speed sort streams can be incorporated into a single bio-safety cabinet for high capacity, multi-parameter sorting of infected cells. This system requires an operator with a degree of skill similar to that needed for the MoFlo. Another nice feature of this system it that all operations can be monitored and the instrument controlled over the internet. (Biorad is soon coming out with a very basic bench-top cell sorter that is advertised as even easier to use than the Aria series, but is a more limited instrument in terms of parameters)

• A four laser cell flow cytometer such as a BD Fortessa (Approximate cost £220,000)

• A third user-friendly flow cytometer such as a BD FacsVerse, BD Accuri, Millipore Guava or Beckman-Coulter Gallios to facilitate unassisted usage and obtain the capacity needed (~£50,000-£110,000).

• Possibly an Amnis ImageStream Cytometer (~£ 250,000-380,000), which performs high content image analysis in flow, including morphology, fluorescence and quantification. Across Oxford, one is currently managed in by the NDM Experimental Medicine unit and housed in the JR and is used by the TGU, but will not move to the ORC. Image quality is similar to that delivered by a 20x NA 0.7 widefield fluorescence microscope image.

Parisa Amjadi from the KIR has recently negotiated the purchase of equipment for their facility and would be willing to obtain the best possible discounted price for any new equipment.

Note that the purchase of so-called user-friendly devices means that a full time operator is not needed for most uses, and therefore costs of running the equipment are reduced.

In summary the equipment that would ideally be available in any new facility would be:

The current Jenner-operated upgraded MoFlo Cell Sorter plus laminar flow hood

A new FACS Aria 4 laser sorter

The current Jenner-operated Beckman Coulter Cyan cytometer

The current McMichael group-operated Beckman Coulter Cyan cytometer

A third user friendly cytometer (Cyan equivalent) either new of from other users

A new 4 laser Fortessa cytometer

A tissue culture hood.

Costs

The recommendation is that any charges levied by any new facility should be low to encourage uptake by users across campus on the principle that cost of use should not be a barrier to science and that it would be better to have 80% use at low cost than 20% use at high cost. The aim should be to match the lowest available in Oxford to ensure competitiveness, and attract users. Moreover to meet EU funding audit requirements, any recharges need to be based on actual rather than estimated costs.

The current cost-recovery model used by Jenner works, but is predicated on the fact that Jenner operates on grant income where current charges and usage are factored into the funding applications.

This is not the case for all other potential users. Although, grants support the research by the groups migrating from the WIMM, the costs factored into those of the McMichael-associated groups, who currently do not pay for FACS use at the WIMM, are lower than those currently used for Jenner. The charges currently levied by the Jenner service would be incompatible with the level of usage required by some of the incoming WIMM groups. A similar situation arises with the Channon group at the WTHGU who factor the discounted rate of £27 ph into their grants.

Other departments such as Oncology and the Ludwig have a degree of core funding that means that potential users in these departments must choose between spending funds on FACS or using those resources for other purposes, eg consumables for post-docs DPhil students. As a consequence current high charges discourage use, and potential users shy away from research that would require cell sorting or high-end cytometry. OCDEM has also indicated that its usage would increase if charges were lower.

Examples of charges levied by different facilities are:

Jenner

Internal users £70/h.; Non-Jenner users £80/h unassisted/£100/h assisted; discounted price for WTCHGU £54/h assisted, £27/h unassisted.

BRC

£35/h unassisted, £85/h assisted, £100/h for cell sorting.

WIMM

For external users £35/h unassisted, £80/h assisted, £100/h assisted.

KIR

Charges to be determined and is open to setting consistent costs for ORC users through the ORC Flow Cytometry User Group.

Jenner has existing agreements between IAH (now Pirbright Institute) and AHVLA whereby they hold joint meetings and workshops to discuss FACS research, protocols, standardization of methods and future commitments to training etc. These need to be factored into any new core support facility, but such meetings/discussions should be expanded to include the KIR.

The KIR currently subsidises its facility from overheads from grant income. However, as this is limited and the facility and its costs are growing, the KIR will be moving to a charging system, but at as low a cost as possible for maintenance of equipment and staff.

It will be especially important to coordinate charges across campus between any new facility and the KIR.

Funding and sustainability

In addition to a degree of cost recovery from user charges, Mark Middleton, as Director of the Experimental Cancer Medicine centre, has indicated that he would potentially be willing to provide some support if the new facility were to promote the clinical science interface, something that would be readily justifiable, especially if the Ludwig Institute, and Oncology were to take up use of the new facility. Similarly OCDEM may contribute, as would the Dept. of Oncology, in return for access to an upgraded FACS facility on campus. Oncology and have indicated that trading in their 20 year-old FACS Vantage could generate £30k.

Potential contributions from these and any other sources need to be taken into account when preparing a business case for any new facility, but it is widely viewed that usage of any new facility would generate sufficient income to cover service charges, and contribute to salaries of key operators, especially if additional contributions were forthcoming. It was felt that charges aimed at recovering depreciation costs would be too high, but that given targets for future investments, a fund could be established that would attract matching funds from other sources, eg the John Fell Fund.

The major influence on sustainability is whether an ORC FACS facility would attract high use. This is in turn determined by the combination of level of cost (lower charges attract more users), facilities, responsiveness, availability, and competition. As such a potential threat to sustainability of core facilities is the acquisition of cell sorters and flow cytometry systems in labs of major users, which then siphon off use as close neighbours can often take advantage of available time without or with lower cost. This can result in lower use of core and need for higher costs. For example, Oncology/The Gray Institute in the ORCRB is investigating whether it can upgrade its own FACS facility, though discussions with Prof. Gillies McKenna indicated that Oncology would in principle be very supportive of a campus-wide FACS facility. If the core performs well and can respond rapidly to needs then the drive for individual labs to go out on their own will be lower and this pitfall may be avoided. Any ORC user group will need to accept feedback and meet the needs of campus.

Submitted March 30th 2013

Colin Goding, Chair of the ORC FACS review

**Appendix I**

Major FACS Users on the ORC from Summer 2013

**User Location**

The Kennedy Institute The Kennedy Institute

The Jenner Institute ORCRB

The Translational Gastroenterology Unit TDI Building

Andrew McMichael/Sarah Rowland Jones, Seph Borrow Groups TDI Building

High–throughput screening service/TDI TDI Building

Cornall Lab CCMP

Channon Lab WTHGU

OCDEM OCDEM

Minor Users

Oncology/Gray Institute ORCRB

Ludwig Institute ORCRB

SGC ORCRB/TDI

One to one-consultations were held with:

Kennedy Institute

Marc Feldmann, Parisa Amjadi, Michael Dustin

Jenner Institute

Helen McShane, Drew Worth, Gary Strickland

TDI

Andrew McMichael, Sarah Rowland Jones, Seph Borrow

Daniel Ebner

TGU

Fiona Powrie

OCDEM

Patrik Rorsman, Anna Gloyn, Rajesh Thakker, Frederick Karpe

Oncology

Gillies McKenna, Mark Middleton, Jean-Baptiste Cazier, Len Seymour, Mick Woodcock

Ludwig Institute

Xin Lu

CCMP

Richard Cornall

WTHGU

Keith Channon/Deborah Hay, Shoumo Bhattacharya.

Round table discussion held on March 22nd with:

Helen McShane, Andrew McMichael, Seph Borrow, Anna Gloyn, Drew Worth, Daniel Ebner, Parisa Amjadi, Sarah Rowland-Jones.

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| **APPENDIX II: FACS Equipment on the ORC** |
| **Flow Cytometers** | **Details**  | **Housed** | **Current usage** | **Notes** | **Accessible/charges** |
| Beckman Coulter - Cyan ADP | Violet (2), Blue (5), Red (2) plus FSC and SSC | Will be moved to TDI Building 2013 | 70-80% | Currently Housed in WIMM flow cytometry facility  | £35h unassisted/ £80h assisted |
| LSRII (BD) | 3 lasers Blue/Red/Violet15 parameters plus FSC and SSC | Jenner/ORCRB | 80% |  | Yes: andrew.worth@ndm.ox.ac.uk |
| Beckman Coulter CyAn ADP LX | 3 lasers Blue/Red/Violet9 fluorescent parameters in addition to FSC and SSC | Jenner/ORCRB | 20%  | Non-Jenner **£80 ph** unassisted / **£100 ph** assisted.Discounted price for WTCHGU **£54** / **£27 ph** respectively. | Yesandrew.worth@ndm.ox.ac.uk |
| BD FACSCalibur | 2 lasers Blue/Red4 fluorescent parameters in addition to FSC and SSC, 2000 events per second | Jenner/ORCRB |  |  | Yesandrew.worth@ndm.ox.ac.uk |
| FACSCanto (BD) | 2 lasers Blue/Red in addition to FSC and SSC | Ludwig/ORCRB | 30% |  | Yesneil.carveth@ludwig.ox.ac.uk |
| FACSCanto (BD) | 2 lasers Blue/Red in addition to FSC and SSC | Cornall lab/ CCMP | 80% | Fees apply for external users at discretion of R Cornall | Yes richard.cornall@ndm.ox.ac.uk |
| BD LSRII | 3 lasers Blue / Red/ upgrade with Violet | Kennedy |  | 5 years old (might do a laser upgrade with violet)  | Free for Kennedy usershelen.bull@kennedy.ox.ac.uk |
| BD FACSCanto II with HTS | 3 lasers Blue/ Red/Violet8 fluorescent parameters in addition to FSC and SSC | Kennedy | 80% | 5 years oldNo upgrade possibleHTS possible | Free for Kennedy usersparisa.amjadi@kennedy.ox.ac.uk |
| BD LSR Fortessa | Blue/Red/Violet plus Yellow-greenFull configuration 6 colour with violetPlus plate reader | Kennedy  | 80% | Will buy for Oxford (4 laser + HTS possible) | Free for Kennedy usersparisa.amjadi@kennedy.ox.ac.uk02083834491 |
| BD FACSCalibur  | 2 lasers Blue/Red | Gray  |  |  | Noclaire.shingler@oncology.ox.ac.uk |
| CytoF | Advantage, no background up to 50 different metal-tagged antibodiesCan self-label antibodies1 of only 2 in UK | Kennedy/Botnar | Still in testing phase | New and already in Botnar | Free for Kennedy usersParisa Amjadi parisa.amjadi@kennedy.ox.ac.uk02083834491 |
|  |  |  |  |  |  |
| **Cell Sorters** |  |  |  |  |  |
| DakoCytomation (Beckman Coulter) MoFlo Legacy MLS | Lasers: Blue /Red488nm coherent Sapphire 200mW adjustable 633nm HeNe laser with 75mW. 7 parameters plus FSC and SSC4 way sort into tubes or 96 or 384 well plates. Possibility to cool or heat both sample and sorting vessel. | Jenner/ORCRB | 40% | Purchased 2005Manufacturer support to 2014(difficulty in sourcing analog electronic parts)Service contract £6000 pa excluding main laserService call out can lead to £700 charge plus parts | Yes:Non-Jenner £100 ph WTHGU £54 ph |
| FACSAria III | 4 laser cell sorter Yellow/Green plus Red/Blue/Violet | Kennedy | 50% predicted to rise  | Will buy for Oxford  | Free for Kennedy users<parisa.amjadi@kennedy.ox.ac.uk> |
| FACS Vantage | Single usable laser; £25k to restore 2nd laser | Gray |  | 20 years old | Free for Oncology Users |