

Guide to Buck Flow Core services

Scheduling instrument time

Both instruments will generally be available for operator-supported use from 9am-5pm Monday-Friday. Operator support outside of these hours may be available, but is not guaranteed. Use of the instruments without operator support, contingent upon satisfactorily completing 1 to 4 hours of training (depending on the user and the instrument), will be available 24/7/365.

To schedule time on either the Aria II or the LSR II, email HKasler@buckinstitute.org or @Flowcore (flowcore@buckinstitute.org) with the following information:

For both instruments:

- Your name and your PI or company's name
- Which instrument you need
- The date and start/finish times
- Will you need operator support?

For the Aria only:

- Will you be culturing the sorted cells, i.e. do you require sterility?
- Is your sample potentially biohazardous? If so, why? **NB:** all human or primate-derived cell lines fall into this category.
- What type of cells you will be sorting and/or what nozzle will you need? (If unsure, ask us how to determine this) • (optional) What colors will you be using and what population(s) will you be collecting? This information will save time (=money) by allowing us to set up experimental templates in advance.

You can check for availability of each instrument by viewing its public Google calendar. These calendars will eventually be embedded in the Flow Core website, but for now you can access them with the following web links:

To view in any web browser, paste the link below into the web address window:

For the Aria II:

https://calendar.google.com/calendar/embed?src=chlls6j90dijqfigmte98ooivc%40group.calendar.google.com&ctz=America/Los_Angeles

For the LSR II:

https://calendar.google.com/calendar/embed?src=kml4ookjach4hcumm82cl3sr3k%40group.calendar.google.com&ctz=America/Los_Angeles

To add to your Google or Outlook calendar app, right-click on “other calendars”, select “open calendar” and paste the link below into the “internet calendar” box:

For the Aria II:

<https://calendar.google.com/calendar/ical/chlls6j90dijqfigmte98ooivc%40group.calendar.google.com/public/basic.ics>

<https://calendar.google.com/calendar/ical/kml4ookjach4hcumm82cl3sr3k%40group.calendar.google.com/public/basic.ics>

Where we are located

The flow core is located on the third floor of Building 3, in room 3-324. In addition to the instruments, the room is equipped with TC hoods/incubators, a benchtop centrifuge, an inverted fluorescence microscope, and plastic ware to allow for processing of samples. Lab coats and gloves are required while working in this room, and eye protection may be required during biohazardous sorting operations. The core director's office is located in room 3-337 in the nearby office suite.

Core recharge rates

For internal Buck users:

The FACS ARIA II will be billed at **\$65/hr.** for self-operation and **\$115/hr.** for operator-assisted use.

The LSR II will be billed at **\$45/hr.** for self-operation and **\$95/hr.** for operator-assisted use.

Training for self-operation will be charged at the operator-assisted rates for each instrument.

Consultations about experiment/panel design and whether or not we can do what you need to do will be **free for the first 30 minutes.** Feel free to inquire or drop by the core director's office, 3-337

For external users:

We welcome business from external clients in either academia or industry, however recharge rates for these services are still being determined. Please inquire by emailing flowcore@buckinstitute.org.

Instrument capabilities

BD FACSAria II cell sorter:

- This instrument, upgraded and refurbished by Cytek, includes four lasers at 405nm, 488nm, 561nm, and 640nm. The filter set allows for simultaneous analysis of 14 color channels, as detailed in the diagram at the end of this document.
- With the smallest nozzle size (70µM), suitable for leukocytes or other small cells, we can typically process up to 15,000 events/second (= 5.4×10^7 /hr.) with >80% sort efficiency. Larger nozzles (up to 130µM) can handle larger cells, but will require lower event rates. Up to four populations can be collected simultaneously into tubes from 1.5-15mL volume. Sorting into 96-well plates is also supported.
- The Aria II is housed in an externally ducted BioBubble Class I BSC, which is compliant with ISAC guidelines for sorting all P2-level biohazardous materials, including primary human/primate cells and cells infected with replication-defective lentiviral vectors.

BD LSR II analyzer:

- This instrument has the same four laser wavelengths and 14 emission filters as the Aria II, as detailed in the diagram at the end of this document. Up to 20,000 events/second can be collected.
- A high-throughput auto sampler will support automated analysis of up to 400 samples/hr.

For both instruments:

Please consult the visual guide at the end of this document for the specific filters and examples of common dyes that are usable. If your dye/fluorescent protein is not listed, ThermoFisher has a very useful resource for evaluating dye performance here:

<http://www.thermofisher.com/us/en/home/lifescience/cell-analysis/labeling-chemistry/fluorescence-spectraviewer.html>. Refer to the diagram at the end of this document for the laser wavelengths and emission filter bands. For the lasers, select these numbers from the drop-down menu in the “light sources” tab. For the filters, select “custom filter” from the drop-down menu under “emission filters”, then input the first and second numbers into the boxes that pop up. Finally, select one of the many listed molecules from the drop-down menu in the “fluorophore” tab, and observe how well the excitation and emission wavelengths coincide with the laser and filter wavelengths. The height of the curve will be proportional to the efficiency of detection. If you are still unsure if your dye/protein can be detected, please feel free to contact us (@Flowcore) for more information.

Contact us

- For scheduling instrument time, training, etc., please email @Flowcore (flowcore@buckinstitute.org) to send your inquiry to all flow core staff.
- For inquiries about experimental design, instrument capabilities, or core service charges, please contact Herb Kasler, the flow core director, via phone (415-209-2056 desk/510-847-6990 mobile) or email (HKasler@Buckinstitute.org).
- In case of emergencies during self-operation, please contact either Herb Kasler (510-847-6990 mobile) or Cori Conner (Cconner@buckinstitute.org)

Buck Institute FACS Aria II and LSR II Optical configuration

(14 colors total, both instruments have the same lasers and filter set)

