Cyto-Spin Instructions

- 1. You must have previously filled out a project information sheet.
- 2. Turn on main switch (on front right hand side)
- 3. If necessary balance by picking up front of machine and GENTLY replacing on counter.
- 4. Open chamber lid (release button on front).
- 5. Pop up metal knob on centrifuge cover and remove lid.
- 6. Insert Superfrost Plus, right side up, into metal clip.
- 7. Place filter, beveled side up onto slide.
- 8. Place plastic cyto-funnel on top of filter.
- 9. Lock clip into place.
- 10. Prepare cells; Wash cells with PBS 2x to remove media. Add an equal amount of fixative and mix up cells gently.

Recommended fixatives are:

- a. Saccomano-good morphology
- b. 95% ETOH-good antigenicity
- c. 20-22% BSA- good antigenicity
- 11.Put 6-8 drops in cyto-funnel, close plastic lids <u>tightly</u>. MAKE SURE THAT THE SLIDES ARE BALANCED IN THE CENTRIFUGE!!
- 12. Replace centrifuge lid, making sure rubber gaskets are in correct placement.
- 13.Lock down by pressing on metal knob.
- 14. Close chamber.
- 15. Push the <set time> button, enter time in minutes (7 is a good starting point), press <enter>.
- 16. Push the <set speed> button, enter desired speed in RPM (700 is a good starting point), press <enter>.
- 17. Press start.
- 18.Once spun, pull out clip assembly and open clip. Pull out slide and filter together. Lift filter straight up from slide. Air dry until mostly, but not completely dry. Place slide in PBS. Can hold up to a week before staining. If using Saccomano fixative, remove with a soak of 95% ETOH for 30 minutes before staining.
- 19. Clean up; Throw filter into biohazard container. Soak reusable parts in 10% bleach solution for 15 minutes. Remove metal clips immediately. Wash in hot soapy water then distilled water.
- 20. Enter your 4-digit account number onto the sign-up sheet and number of spins used.