STANDARD OPERATING PROCEDURE

CytoFuge2 Cytocentrifuge System

PURPOSE

This document describes the operational procedures for the CytoFuge2 cytocentrifuge system (CytoFuge2). This document offers users a brief step-by-step guide on how to operate the CytoFuge2.

The CytoFuge2 is a microprocessor-controlled cell preparation system that uses centrifugal force to deposit cells onto microscope slides. This bench-top cytocentrifuge provides an easy and quick tool to concentrate cell suspensions onto microscope slides with a level of cell recovery, clarity and detail that cannot be achieved with direct smear techniques. Samples are centrifuged in reusable or disposable gasket-sealed chambers (Cell Concentrators), or in disposable chambers (Filter Concentrators) that include a filter material to absorb and capture suspension fluid during cytocentrifugation. Speeds from 600 rpm to 4400 rpm and cycle times from 2 minutes to 10 minutes can be selected.

SCOPE

The procedure applies to spinning of cell suspension samples with disposable filter concentrators (assembly of reusable cell concentrators is not covered).

SAFETY

Cytospinning of samples on the CytoFuge2 must only occur after the approval of an associated project in PPMS. Any projects to be run on the CytoFuge2 involving hazardous chemicals must have appropriate approval. OGTR requirements for safe work in a PC2 laboratory apply. When potentially biohazardous materials are used, it is required that the CytoFuge2 is operated in a biological safety cabinet.

For general Biosafety information, refer to Safe Work Procedure WIMR-SWP-WHS-GEN-16.01, “Using a fluorescence or laser scanning microscope”:
https://sydneyuni.atlassian.net/wiki/spaces/WIF/pages/768016621/Manuals+Protocols.

The operator must exercise caution before and during operation of the CytoFuge2:

- Before turning on the CytoFuge2, inspect the instrument for cracks or any physical damage to the housing, cover, and rotor.
- Maintain a 30 cm (12 inch) clearance free of obstructions around the equipment during operation.
- Always operate the system with all shields and doors in position and secured to avoid injury.
- Wear Personal Protective Equipment (PPE) such as gloves, eye shields, and lab coats.
- These hazardous materials are prohibited to be used within the CytoFuge2: flammable or explosive materials and materials which could react chemically with sufficient vigour to cause a hazard.

TRAINING / COMPETENCIES

All personnel require training prior to independent operation of the instrument. Training is conducted by imaging staff and competency demonstration is necessary before authorisation.

Competency is assessed via demonstration of independent instrument operation, in conjunction with verbal explanation of all aspects of operation and troubleshooting common faults. After training is carried out, a competency quiz is to be completed and passed before instrument access is authorised by imaging staff. All instrument operation is to be conducted by trained operators.

EQUIPMENT & SUPPLIES

The CytoFuge2 is located in the shared Prep Lab J.2.06, Level 2 WIMR.

OUTLINE

PROCEDURE

1. Sample requirements and preparation

1.1 Sample volumes

Use the following guidelines for sample volumes in each of the CytoFuge2 concentrators:

	Volume Range	Optimum Volume Range
Filter concentrator	50 – 300µL	100 – 300µL
One-well cell concentrator	300 – 1,600µL	400 – 800µL
Three-well cell concentrator	50 – 400µL	100 – 200µL

1.2 Concentration

The approximate cell concentration of the specimen should be established before slide preparation on the CytoFuge2.

The following is a general guideline for sample concentration based on an average cell diameter of 10µm:

Sample Concentration	Recommendation
500-1500 cells/µl	Use optimum sample volume
< 500 cells/µl	Pre-concentrate sample
>1500 cells/µl	Dilute sample

2. Audible indicators

2.1 Normal function codes

Sound	Meaning
2 medium beeps	Sounds on power up; instrument ready
3 short beeps	Cycle completed per specified operating parameters
Chirp	Start or stop command recognized by the microprocessor

2.2 Malfunction codes

Error Indicator	Error
1 long beep followed by: 2 short beeps	Lid opened during operation
1 long beep followed by: 1 short beep	Rotor failed to reach selected rpm within allotted time
1 long beep followed by: 4 short beeps	Insufficient power to maintain rpm

Note:

The system will beep continuously if one of the following conditions are present:

- Centrifuge is over operating temperature
- Short circuit of the motor drives, fan, or solenoid
- Reduction in the availability of electrical power
- Short-term power failures

To stop the beeps, press the **stop** button 

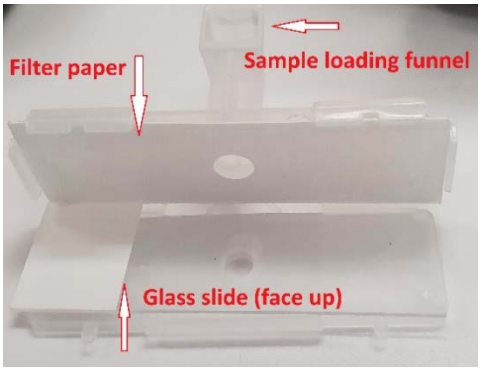
3. Operation of CytoFuge2

Note: Do not operate the CytoFuge2 or its rotor below the minimum operating temperature (10°C).

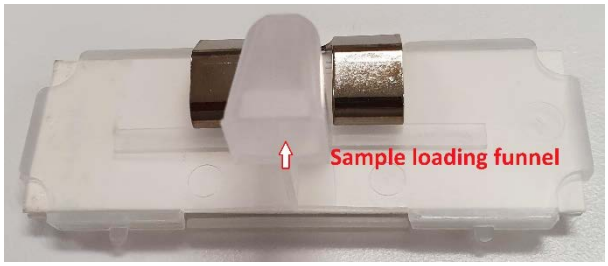
3.1 Assemble Cell and Filter concentrators

Assemble the filter concentrator as shown below (**A**), and then secure the concentrator assembly by sliding in the reusable clamp from the back of the sample loading funnel (**B**).

A



B



3.2 Opening and closing the cover

The electrically operated cover interlock mechanism prevents operation until the cover is completely closed and locked and prevents the cover from being opened while the centrifuge is in operation. When the cover is completely closed and locked, then an operating cycle can be initiated.

The centrifuge has a manually operated latch that holds the cover down after spinning is complete. The interlock is automatically released at the end of the operating cycle, or by pushing the **Stop** or **Open** button. Squeeze the black latch pieces together to open the cover.

Caution!

- Never operate the CytoFuge2 without the rotor cover in position.
- The cover interlock bypass is for emergency use only. Disconnect the power cord of the external power supply from the electrical outlet and ensure the rotor has come to a complete stop before using the interlock bypass. If the equipment is not used correctly, safety can be impaired.

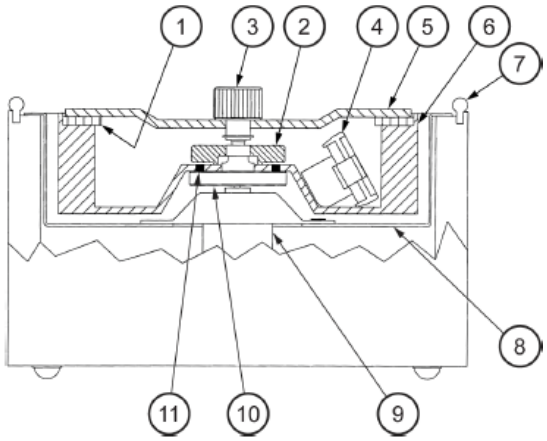
3.3 Screw off the rotor lid

Turn the lid nut counter clockwise while holding the rotor itself to prevent turning. Take off the rotor lid.

3.4 Access the Rotor

The rotor has four positions, allowing two or four concentrators to be centrifuged.

Cross-section of the CytoFuge2



1. Rotor Gasket
2. Rotor Hold-Down Nut (3-lobed)
3. Cover Nut
4. Cell Concentrator (shown in rest position)
5. Rotor Cover
6. Rotor
7. Bowl Gasket
8. CytoFuge Bowl
9. Motor
10. Rotor Mount
11. Rotor Indexing Pin

3.5 Install the assembled concentrators

Ensure that the assembled concentrators are installed into the rotor in a balanced arrangement (Empty concentrators can be used for balance).

Caution!

Running the centrifuge repeatedly with an unbalanced load condition can cause excessive equipment vibrations and premature equipment failure

3.6 Load the sample

Before loading the sample, first place the assembled Cell and Filter Concentrators in the CytoFuge2 rotor.

Slowly add the sample to the bottom of the funnel. Avoid getting droplets onto the walls of the funnel. Avoid exposing the filter material or the microscope slide to the sample during the loading process. Do not overfill the device.

Note: samples should be processed as quickly as possible following sample addition to prevent cells from settling.

Caution! Addition of excess amounts of liquid (overfilling) to Cell or Filter Concentrator causes the fluid to spin out during centrifugation.

3.7 Close the centrifuge cover (refer to 3.2)

3.6 Spin the Sample

Select the speed and time with the dials on the side of the CytoFuge2.

CytoFuge2 operating parameters generally depend upon the size and specific gravity of the cells to be concentrated onto the microscope slide. The operator should experiment with different settings to achieve optimum performance for specific applications.

The following are general guidelines:

Application	Speed Range	Time Range
Cytology	600 – 1000 rpm	2 – 4 min
Haematology	1000 – 2,200 rpm	4 – 8 min
Microbiology	1600 – 4,400 rpm	4 – 10 min

Note:
- For fragile cells, reduce the speed
- For wet preparations, reduce the time
- For small particles (e.g. bacteria), increase the speed and time

To start the spin, Press the **start** button 

Caution! Do not lift or move the CytoFuge2 during operation to avoid injury to the operator and/or damage to the centrifuge.

4. After operation is finished

4.1 Disassemble

- Unscrew the rotor lid
- Remove the concentrators
- Disassemble the concentrators
- Recover the slides for further processing

4.2 Cleaning

- Clean the reusable camps, outside surfaces, control panel and inner surface or bowl of the Cytofuge2 with a paper towel and 70% alcohol.
- If necessary, 0.1% bleach solution can be used after diluting from 10% bleach with the provided measuring cups and tap water. Wipe off any residual bleach with a clean paper towel (dry, or wet with either 70% ethanol or water).

Caution!

- Do not spray cleaning solutions directly onto the centrifuge bowl or housing. Overspray of cleaning solutions can reach the motor bearings or internal circuitry, causing harm to the electronics.
- Do not expose CytoFuge2 and its rotor to strong or concentrated acids, bases, esters, aromatic or halogenated hydrocarbons, ketones, strong oxidizing agents, or environmental influences, including natural ultra-violet radiation.

DATA & RECORDS MANAGEMENT

This system does not come with a computer and therefore bookings are necessary.

REFERENCES

Hardcopies of both quick manuals provided by the instrument vendor are available in the cabinet underneath the bench where the instrument is located. E-manuals are also available on the Imaging Website: https://sydneyuni.atlassian.net/wiki/spaces/WIF/pages/768016621/Manuals+Protocols.

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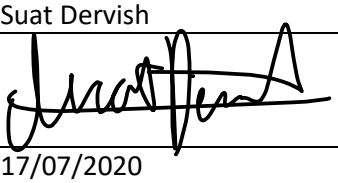
Signature of Approval Authority:	
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REVISION HISTORY

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