

# Equipment Overview

## 5<sup>th</sup> floor CTW and beyond...



Revised 8/3/18

# Purpose

- To inform you of equipment available for common use on the floor and beyond
- To review protocols for use
- To solicit your help in maintaining/overseeing specific equipment items on the floor
- To inform you that use of some pieces of equipment and/or services requires that you cite specific grants in publishing data that was obtained using any this equipment

# How does this floor help your research?

- Provide liquid nitrogen, dry ice for general use.
- Supports maintenance contracts on centrifuges, autoclaves, film developer (and regular service), scintillation and gamma counters
- Supplies reagents for dishwasher, film developer, water purification system
- Maintains and oversees common use equipment

# How can you help?

- Sign log books (important for maintenance contracts and for general monitoring of equipment)
- Receive training on equipment prior to use to assure that equipment is kept in good operating condition
- Keep things clean
- Step up to the plate to volunteer to oversee a piece of common use equipment
- Be regular monitors yourselves, if you don't like something you see, tell the person using the equipment
- Cite appropriate grants when publishing your findings

# The COCVD Metabolic Phenotyping Core

- Located in Biopharm DLAR
- Operated by W. Katz, see Dr. Katz for protocols, scheduling use, design issues
- Supported through the Center of Biomedical Research Excellence on Obesity and Cardiovascular Diseases (COBRE)
- Anyone who uses these chambers for studies must cite the NIH grant (P30 GM127211) on publications.

# The COCVD Metabolism Core

- The TSE LabMaster Indirect Calorimetry System monitors energy expenditure, activity, and food and water consumption of mice in individual chambers.



# Standard Protocol - acclimation

1. We recommend that lean and fat mass be determined by EchoMRI at the beginning and end of the experiment.
2. Investigator weighs mice and places them in acclimation chambers prepared by core personnel.
3. Food and water bottles are weighed at the start of acclimation and the following 1-2 days to confirm that mice are eating and drinking.
4. Mice remain in acclimation chambers for 1-2 weeks, depending on age and obesity. Investigator checks mice daily during acclimation.



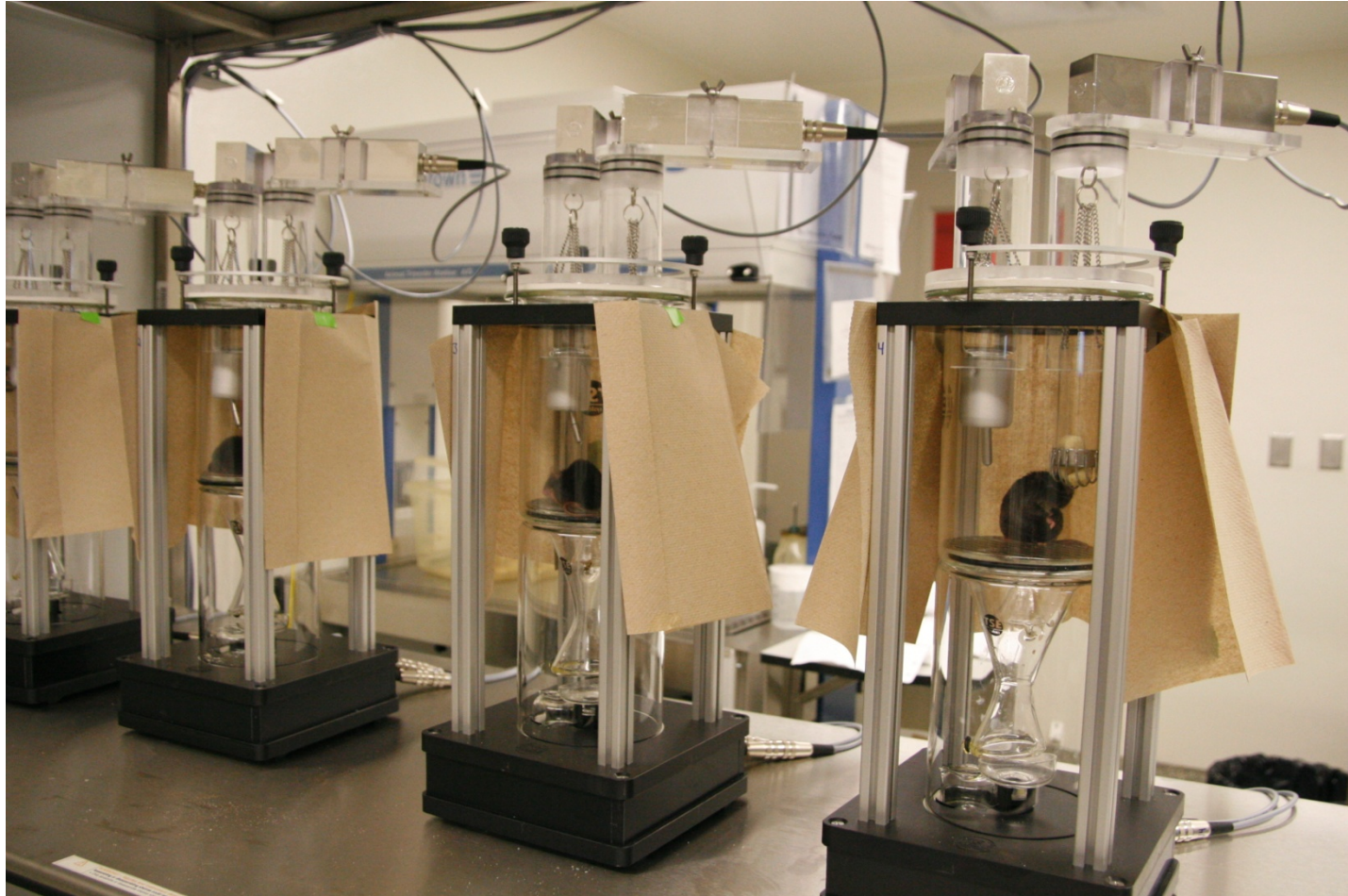
# Standard protocol - recording

1. Core personnel weigh mice and transfer them to recording platforms on Monday morning.
2. Core personnel check mice daily during recording period.
3. On Friday, investigators and core personnel weigh mice and return them to standard cages.
4. Investigators assist with cleanup and perform EchoMRI.





The TSE Phenomaster Metabolic cage system collects urine and feces while simultaneously recording food and water intake.



# Standard Protocol - acclimation

1. Investigator weighs mice and places them in acclimation chambers prepared by core personnel.
2. Food and water bottles are weighed at the start of acclimation and the following 1-2 days to confirm that mice are eating and drinking.
3. Mice remain in acclimation chambers for 3-7 days. Investigator checks mice daily during acclimation.



# Standard protocol - Collection

1. Core personnel transfer mice to collection chambers.
2. Core personnel transfer samples each day to containers provided by investigators, and store or deliver as arranged.
3. After 1-3 days, investigators and core personnel weigh mice and return them to standard cages.
4. Investigators assist with cleanup.



# EchoMRI

- Located in B014, BBSRB,
- Noninvasively quantifies fat/lean mass and total body water in conscious mice
- Scheduling through Sharepoint calendar maintained by Wendy Katz
- This equipment is part of the COBRE, you must cite grant P30 GM127211 when publishing data after using this system



**Figure 1.1: EchoMRI-5000™**

# 5<sup>th</sup> floor microscopes

General Policy: Each microscope has filter controls etc in slightly different places.

The newer Nikons have a special flip-down or slide-in lens for the 4x objective.

➤ If you are using a particular microscope for the first time, ask one of the administrators for an orientation.



# Dissecting Microscope with imaging software: 551



- Dissecting microscope with camera and imaging software for en face quantification of atherosclerosis
- Cassis lab, please see Sean Thatcher for use

# 551: Upright with fluorescence

12 MP color camera.

Objectives:

- 4X Plan Fluor
- 10X Plan Fluor
- 20X Plan Apo
- 40X Plan Apo
- 60X, 100X\*

\*by prior arrangement



Motorized stage with image stitching software

Maintenance and administrative issues:  
Wendy Katz

# Additional objectives

- 60X Plan Apo
- 100X Plan Fluor  
(both oil objectives)

Wendy has them.



For use on *upright* (Eclipse 80i) microscope

Make arrangements with Wendy 24 hrs in advance

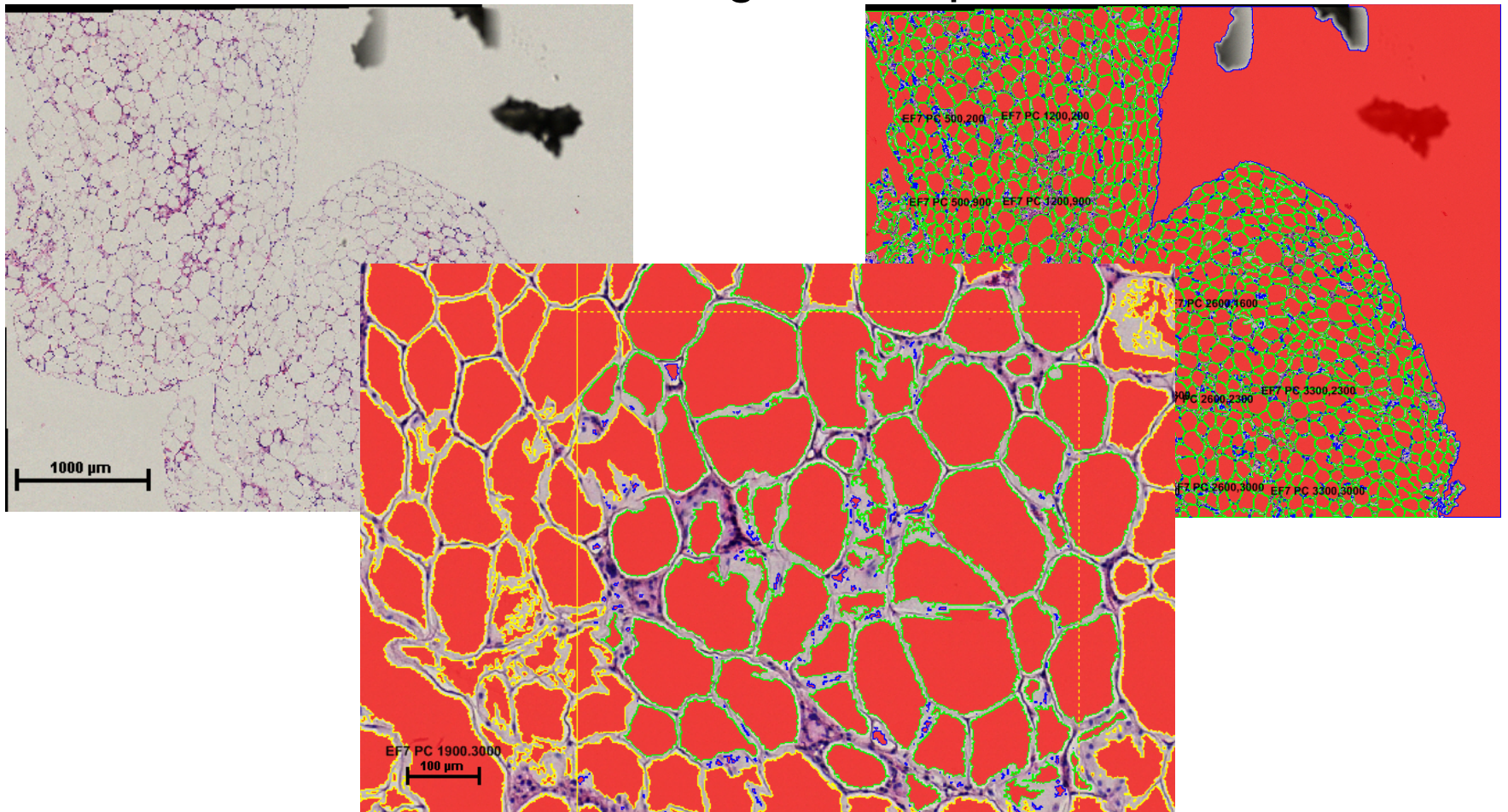


# 551 Nikon Eclipse 80i

- This microscope is primarily for high resolution imaging.
- Please sign up to use the microscope.
- Please move the objective to 4X to insert slides and remove slides from the stage.
- Please do not remove objectives from the microscope..
- The 40X objective is NOT an oil objective. The 60 X and 100X are. If you use oil on your slide do NOT use the 20 or 40X objectives as the oil will ruin the objectives.
- If you aren't sure ASK.....

# Motorized stage in 551

- Joystick control
- Rapid navigation options in software
- Permits automated stitching of multiple fields



# 599B: Inverted with fluorescence

Monochrome camera.

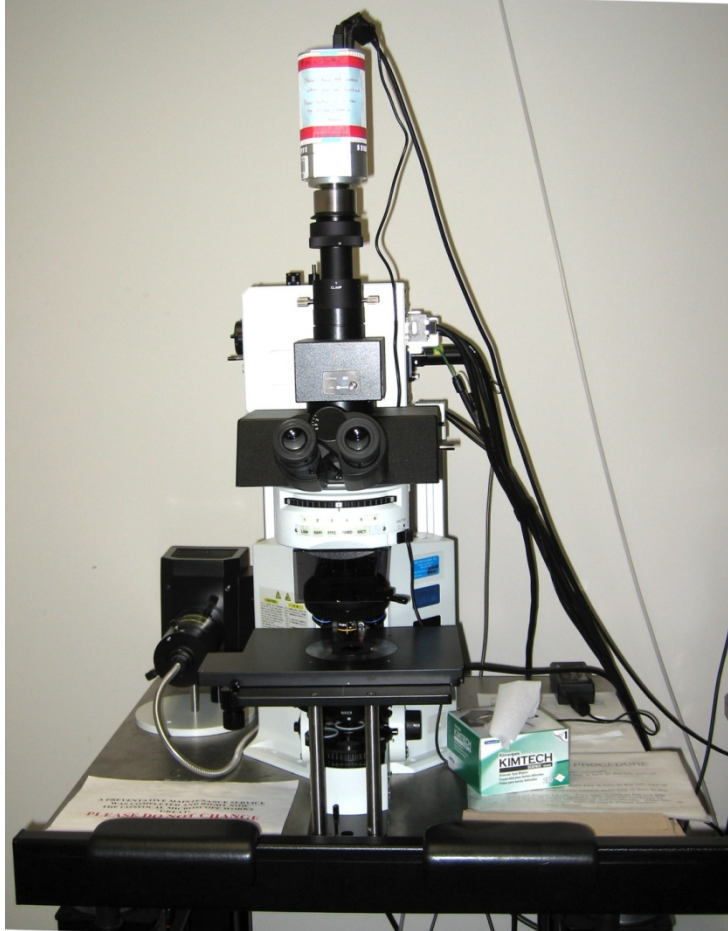
Objectives:

- 2X Plan
- 4X Plan Fluor
- 10X Plan Apo
- 20X Plan Fluor
- 20X Plan Apo
- 40X Plan Fluor



Maintenance and administrative issues:  
Wendy Katz

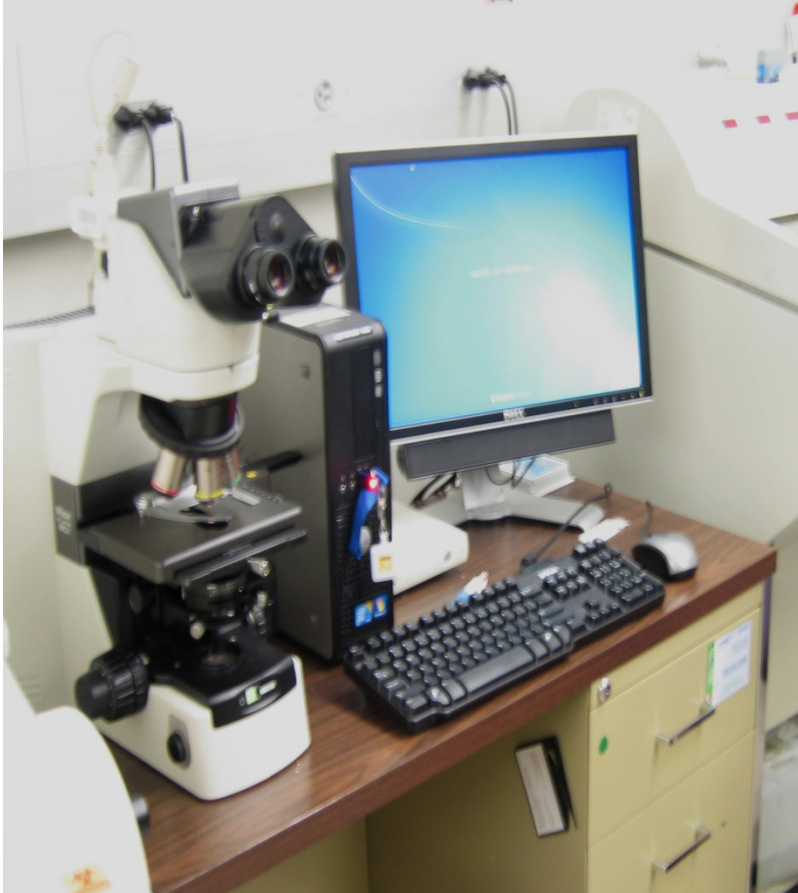
# 599B: Confocal



- BX61WI, Olympus microscope with bio-imaging camera
- Operating instructions next to microscope.
- Turn off switch at the end of use.
- Some maintenance issues on this camera: computer has some error messages, one monitor not working well.
- Contact Changcheng Zhou or Se-Hyung Park for use

# Microscopes: 564

## Nikon Eclipse 80i (*COBRE Core*)



- Color camera
- Objectives:
  - 4X Plan Fluor
  - 10X plan Fluor
  - 20X Plan Fluor
  - 40X Plan Fluor

Administrative and maintenance issues: Wendy Katz

# 564 Nikon Eclipse 80i

- This microscope is primarily for imaging tissue sections and quantification.
- Please sign up to use the scope.
- Nikon NIS Elements Software for quantification (athero, renal....).
- Please use this scope for quantification rather than the scope in 551.
- Please move the objective to 4X to insert slides and remove slides from the stage.
- Again if you have a question ASK.....

# 564: Upright (Eclipse 55i)

Located between embedding center and microtome.

Color camera.

Objectives:

- 4X Plan
- 10X Plan
- 20X Plan
- 40X Plan
- 100X Plan (Oil)



Administrative and maintenance issues: Wendy Katz

# 564: Inverted

Located between embedding center and microtome.

Color camera.

Objectives:

- 2X Plan
- 4X Plan Fluor Phase
- 10X Plan Fluor Phase
- 20X Plan Fluor Phase
- 40X Plan Fluor Phase



Administrative and maintenance issues: Wendy Katz



# Microscopes

- For use and training, contact Dr. Katz and she will refer you from there with the exception of the confocal scope, contact Dr. Zhou.
- Ask Dr. Katz about which of these scopes are part of the COBRE, and require citing the grant
- Please email Wendy when you use a microscope. Quantifying usage helps us allocate maintenance costs.

# 564: Cryostat

Leica (Cassis lab)



Administration and training, Sean Thatcher

# Blood Pressure

- Visitech system maintained and operated by Cassis lab. See S. Thatcher for use.
- Coda8 systems operated by Daugherty lab.
- Radiotelemetry: in BBSRB DLAR, have 20 platforms (16 in use currently), implants owned by Cassis lab. See L. Cassis for use.

# Scintillation/Gamma Counters



- Located in 564 (2 gamma, 1 scintillation) and also in freezer room (1 scintillation)
- Please use log books
- Please do not leave your samples in the counters or on the benches within this area

# Centrifuges: 530



- 2 Ultracentrifuges with multiple rotors in cabinet
- 1 TL100 ultracentrifuge with rotors in cabinets
- Several mid-speed centrifuges in freezer rooms
- Please use log books
- If you do not know how to use the ultracentrifuges, please ask someone for instruction

# Miscellaneous equipment in room 564



- Imaging station
- Nanodrop
  - Please sign the log book.
  - If you have a question ask Analia Loria.
- Real-time PCR located on bench (BioRad Real-time CFX PCR, 30 min. runs)

# Room 564 Kodak Gel Imager

- If you are a new user for this system, please talk to Sean Thatcher to get a short tutorial.
- Please write in the LOGBOOK in order to monitor use.
- For users, please use your Link Blue ID and password to log into the system.
- In order to save your images, please do the following:
  - 1.) Go to the Start Menu
  - 2.) In the “Search Programs and Files” dialog box, please type the following:  
[\\ukhcddata\share\kodak](#) gel imager
  - 3.) Once the file folders can be seen, right click your lab’s file folder and drag to the desktop and create a shortcut to save your images.

# Kodak Gel Imager (cont.)

- When you open the Kodak gel software for the first time, it may ask you to pick a file for Standards. In order to do this, press “OK” and go to the C: drive and find Program Files. Then, find the Kodak MI file folder and then go to the MI Application. In this folder, you should find a file folder called Standards. Open it, and choose any of the files to load into the program. It does not matter which file you choose.
- Now, in order to recognize the camera, go to the file tab and choose “Select Digital Camera.” In the first line, there is a down arrow. Press it and find the item that says “IS 4000 MM”.
- Once you have that, then go back to the file tab and select “New IS4000 Capture”



# Kodak Gel Imager (cont.)

- The camera settings should then load and you should be able to capture an image. After you capture an image, save the image on the desktop using the shortcut that you have created. These images will be a .bip file which can only be used within the Kodak MI program. To create a JPEG or TIFF file, go to the File tab, look for “Export Data” & then go to “Image”. Then save your image by using the same or different file name & choosing a file format via the down arrow.
- Please save your gel images in the file folder designated for your lab. You will then be able to see, copy, or print these images from any computer on the UK network but you will not be able to write on them!

# Kodak Gel Imager (cont.)

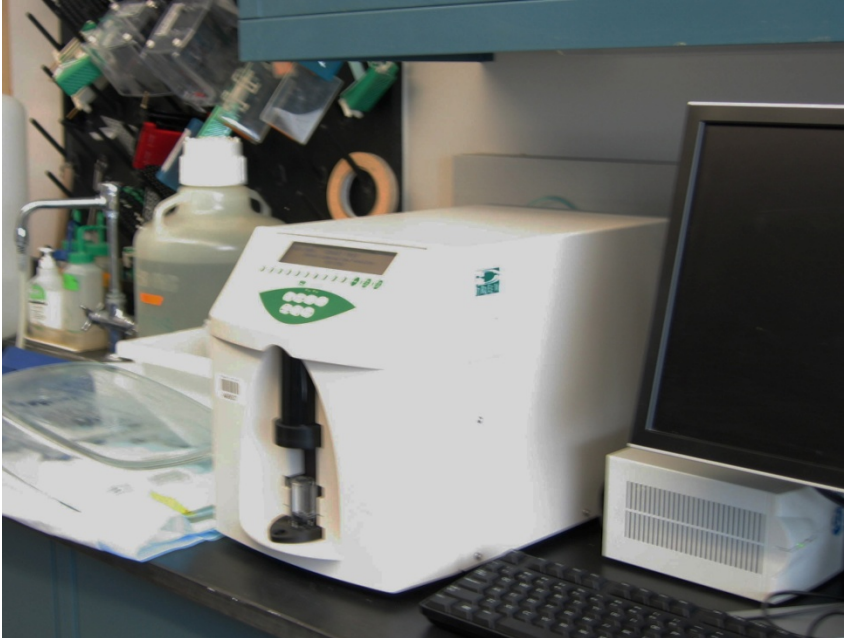
- In order to see your images on your desk computer, please do the following:
  - 1.) Go to the Start Menu
  - 2.) In the “Search Programs and Files” dialog box, please type the following:  
[\\ukhdata\share\kodak](#) gel imager
- **Never, never use a thumb drive for copying your data while using the gel imaging system!!**
- If you have any problems, please contact Sean Thatcher at [SeanThatcher@uky.edu](mailto:SeanThatcher@uky.edu)

# Autoclaves, dishwasher, water purification, cell storage tanks, liquid nitrogen/dry ice



- All located in 526
- Cell storage tank is for common use and hooked to liquid nitrogen (do not disconnect and use this liquid nitrogen)
- 2 liquid nitrogen tanks, one in 526 and one in 518. Tank in 526 is for cryo storage system. Tank in 518 is for common use, do not fill large cell storage containers from these
- Autoclaves, tested monthly by V. English, report to K. Dodridge if problems
- Dishwasher, reagents supplied by K. Dodridge, report to K. Dodridge if problems
- Water purification, tanks changed on regular basis, do not leave unattended containers in this room, report to Karen Dodridge if problems

# Hemavet 950 FS



- Moved to 595, Cassis lab
- Sample data output on right

Sample ID Control blo  
Test No 21  
Species MOUSE  
Date 8/29/2012  
Time 9:33 AM

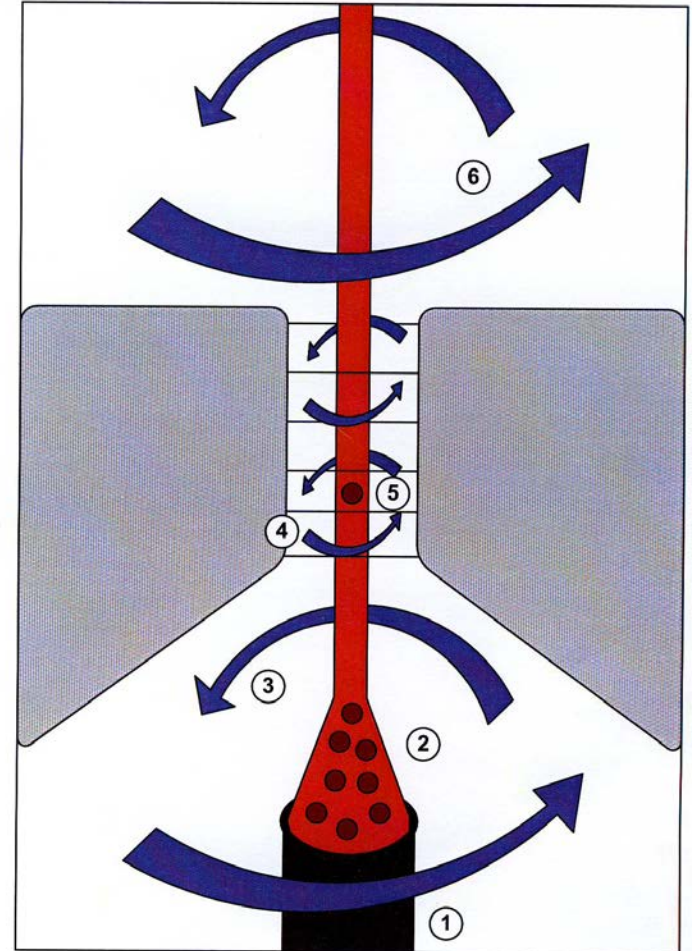
WBC	K/uL	19.04 H
NE#	K/uL	11.18 H
LY#	K/uL	6.71
MO#	K/uL	0.12
EO#	K/uL	0.25 H
BA#	K/uL	0.78 H
NRBC#	K/uL	
NE%	%	58.72 H
LY%	%	35.24 L
MO%	%	0.65
EO%	%	1.32
BA%	%	4.08 H
NRBC%	%	
RBC	M/uL	8.97
HB	g/dL	15.8 H
HCT	%	53.5 H
MCV	fL	59.6
MCH	Pg	17.6
MCHC	g/dL	29.5 L
RDW	%	21.8
RETIC#	M/uL	
RETIC%	%	
PLT	K/uL	703
MPV	fL	7.7
PDW	%	0
PCT	%	

# Features of the Hemavet

- 6-programmed species keys, but can be adapted for other blood specimens (Bison, Calfnote2, Camel<sup>2</sup>, Cat, Cow, Deer<sup>2</sup>, Dog, Elephant<sup>2</sup>, Elk<sup>4</sup>, Ferret<sup>2</sup>, Foal<sup>2</sup>, Goat<sup>2</sup>, Guinea Pig<sup>2</sup>, Horse, Llama<sup>2</sup>, Monkey, Mouse, Pig, Pre-Dilute Mouse, Pre-Dilute Rat, Rabbit, Rat, Sheep)
- 20 µLs of whole blood needed per sample
- Runs 26 samples in 1 hour
- Multi-Controls will be run once per month (Calibrations will be done every 6 months)(ProbeClenz will be run once per week only during use)
- System is setup to run a clean cycle everyday at 6:30am.

# Features of the Hemavet

- Uses focus flow design™
- Constricts the sample flow to 15 microns providing single cell analysis



# Guidelines for using the Hemavet 950FS

Location of the Hemavet (Room 595, Charles T. Wethington Bldg.)

PI in control of the Hemavet is Dr. Lisa Cassis  
Contact person for quality control and maintenance is  
Victoria English.

Please follow the procedures listed below in order that  
the hemavet works properly.

- 1.) Contact Victoria English via email ([vicki@uky.edu](mailto:vicki@uky.edu))  
24 hours prior to running your samples. This will  
ensure that the instrument is ready for samples.
- 2.) Make sure that all solutions for the hemavet are  
properly filled before running samples. If any solutions  
are empty, please contact Victoria English.

## **Guidelines for using the Hemavet (cont.)**

3.) Make sure that all blood samples contain some form of an anti-coagulant. You may use heparin, EDTA, or sodium citrate on samples at the time of collection. We make up a 0.2M EDTA stock and then place 20 $\mu$ Ls into each tube prior to blood collection (final concentration is 4  $\mu$ M). You will need at least 20  $\mu$ Ls of blood per sample for reading on the hemavet. There are also vials that contain EDTA or heparin for blood collection that you may purchase through Fischer or other bio company.

4.) Place samples on rocker prior to reading samples on the hemavet (located next to hemavet).

5.) Please be sure that the software is communicating with the hemavet. There are instructions next to the instrument to ensure that it is working properly. Please follow these procedures. Procedures are also listed at the bottom of this section.



## Guidelines for using the Hemavet (cont.)

6.) Once communication is complete, press #2 on the Main Menu and the black carriage will go in and the probe will go 2 inches down. Once you have your sample inside the unit, press the #2 key again and the probe will go all the way down into your sample. If everything looks okay and you have enough blood in the sample tube, press the “mouse” button to analyze your sample.

7.) After completion of your samples, transfer data to Excel and save on the desktop. Data then may be placed on a thumb drive.

8.) Once data is collected, make sure to fill out the log book (located on top of the hemavet), with your name, date and time, and number of samples that were run through the hemavet. Please make sure to run a **clean** cycle after you have run all of your samples.

## **Guidelines for using the Hemavet (cont.)**

9.) Control samples and calibrations will be done on a monthly or six-month basis, respectively. If you need to evaluate these results, they will be in the notebook in a drawer labeled “hemavet” below the instrument. Please ask Vicki where to find these results.

10.) Lastly, the hemavet will have hours of operation from 9 am-4:30 pm Monday through Friday (this is non-negotiable). We will not have individuals coming into our lab at night or during the weekend. This is to make sure that equipment is used properly and that someone can help if a problem should happen to occur.

If you have not used the hemavet, please find Victoria English and she will go through the proper procedures. Her contact information is

Victoria English

Room 592, Charles T. Wethington Bldg.

Phone number 859-218-0211 (office) or 218-1402 (lab)

E-mail: [vicki@uky.edu](mailto:vicki@uky.edu)

## **Guidelines for using the Hemavet (cont.)**

To use the hemavet, please do the following:

1. Log in as lclab followed by the password "Medicine1"
2. Click the HemavetDMS.exe file and the program should initialize successfully.
3. Before running samples make sure the hemavet is communicating with the computer.
4. Push "9" on the hemavet, followed by "7" (Diagnostic menu), then "3" (DMS menu), and "1" (initialize). Finally, push "0" (3 times) to get back to the main menu.
5. Finally, press start button (green arrow) on hemavet computer program and then press "mouse" on hemavet to start collecting blood samples.

Edited the protocol on 1/19/2016 due to error message 9037 092-057-000-04/H 653 STMCTL. *This error was due to the black carriage getting stuck or being non-responsive.*

# Equipment oversight: Step up!

- Autoclaves: V. English (Cassis lab)
- Cell storage common tank: Vicki Noffsinger (de Beer lab)
- EchoMRI: Wendy Katz
- Hemavet: Victoria English (Cassis lab)
- Confocal: C. Zhou
- Dark room: Z. Guo
- Ultracentrifuges: Vicky Noffsinger (DeBeer lab)
- Water purification: Karen Dodridge
- Dishwasher: Karen Dodridge
- Scintillation/counters: M. de Beer, V. English (Cassis lab)
- Nanodrop: Analia Loria
- Kodak Gel Imager: Sean Thatcher
- Cryostats: Sean Thatcher (Cassis lab)
- Scopes/embedder/image stitcher in pathology area: Wendy Katz
- Fluorescence scope in 599B (confocal room): Wendy Katz
- Cold rooms: Karen Dodridge
- Real-time PCR in 564: Preetha Shridas