MULTI-HEADED MICROSCOPE AND DIGITAL CAMERA

This 4-headed diagnostic bright field microscope (Olympus B40) is a part of the Pathology Core Laboratory, primarily for use by two pathologists (D. Craig Allred and Syed Mohsin) to perform diagnostic evaluation of H&E and IHC slides. This can be used by other members of the Breast Center for reviewing slides with other personnel or collaborators. This is attached to a digital camera (DXM-1200) and a PC with an LCD monitor. The camera can acquire high quality (up to12 megapixel) images as well as provide live image on the LCD monitor (in case of more than 4 users reviewing slides simultaneously). The purpose of this camera is acquisition of high quality images to supplement grants, and manuscripts as well as routine images for presentation for all Breast Center personnel after appropriate training and authorized access.

Instructions for use

Microscope:

This microscope has superior quality objective lenses with 4x, 10x, 20x, 40x and 100x magnifications. Each of the 4 eyepieces is width and focus adjustable for users. The stage is scratch resistant and has an optional attachment for holding slides. The power button for the scope is located on the back of right side of the microscope body, just above the power cord jack.

The scope has several adjustable components:

1. Aperture
2. Condenser
3. Focus control
4. Neutral density filters (three filters)
5. Light path splitter

The **aperture** is located at the base. It can be rotated to adjust an internal aperture that controls the amount of light that can pass from the source bulb (located at the bottom in the base) to the stage. Ideally, *it should remain fully opened*.

The **condenser** is attached on the undersurface of the stage. It can be moved up and down using a dial on the left of the stage and this helps with light defraction. Ideally, the condenser should be kept at the *highest position* to minimize the contrast at tissue edges.

The **focus control** is a secondary aperture that is used for conversion from bright field to dark field microscopy. This microscope does not have dark field filters and cannot be used for that purpose. Therefore, *the focus control should be kept fully opened*.

The **neutral density filters** are inside the base and can be activated using small rotary dials on the right side of the base. For H&E and IHC, these are set with only ND1 active. Introducing these filters dampen the brightness and is usually desirable in

photomicroscopy. Since the digital camera has a powerful system built in the software to adjust the light, these *ND filters should not be changed* until and unless the software is unable to provide optimum white balance.

Light path splitter control is located on the right side of the eyepiece assembly. It is a metallic cylinder that can be pulled in and out at three positions. If pushed all the way in, all the light passes to the microscope eyepieces. When pushed all the way out, all the light passes to the F-mount assembly that holds the camera and nothing is visible in the eyepiece. In the middle position, the light is split equally to eyepieces and camera. This latter position is recommended when using camera.

Digital Camera:

The digital camera is attached to the top of the microscope and connected to a PC, next to the microscope. There are no controls located on the microscope itself and therefore there is no need to touch and move the camera.

In order to use this camera, every user is required to undergo a short training, which can be arranged by contacting the laboratory. Once a person is trained, they are set up by The Breast Center Information Technology section to have personal username and password to log on to the PC. This piece of equipment is available to The Breast Center personnel only.

The software for digital microscopy is called "ACT -1" and this is available as a desktop icon. The microscope should be on and light path splitter at the middle position prior to opening the software. Once open, there is a larger window on the left showing live image and a smaller window on the right to show the last saved image. Below the right image window, there are five set of tabs that contain all the functions. *You do not need to use the tool bar at the top to use this camera*.

General Tips:

1. This is a high resolution camera and if JPEG with high compression is not used, then each image may be 40-80 MB in size.
2. For optimum performance, it is strongly recommended that the images are saved on a remote drive, such as Temp drive.
3. Images saved on the local drives can be transferred by burning on CD (there is a CD-RW on this PC) or USB pen drives. All images on local drives will be removed after 30 days to keep maximum memory available.
4. Since the camera can capture 12 megapixel images, even normal JPEG images can furnish publication quality images, which can then be processed in Adobe photoshop or similar applications with needed print options.
5. A correct white balance and minimal under exposure will provide you with the best raw image. There is a white balance function is the camera software and it is

optimized for your use. It is preferred that you do not use this function routinely, and rely on white balance and contrast in image processing software.

Software Function Tabs

1. **Photo:** It has two functions:
   Mode: Select one of nine desired image resolution and size
   File: Select one of five file formats (3 JPEG, TIFF, and Bitmap)

2. **Save:** It has five functions:
   a. **Image Folder:** Select drive/folder where you want to save images
   b. **File name:** There are two fields to name the image file
   c. **Drive info:** Tells the amount of memory on selected drive/folder
   d. **Default:** Click to save images on a folder on local C-drive of this PC
   e. **Apply:** Click to see last 5 saved images in selected image folder (these are displayed in a row below the live image window on the left of screen

3. **Live:** It has four functions:
   a. **Color balance:** Select if you desire to pre-process color in the images. This is not recommended for routine use.
   b. **Focus mark:** When checked it shows a cross-hairline on live image window. This helps in getting perfect focus which can be monitored on a bar scale on top of the tabs. It shows a number on left and a moving green/red bar on the right. The goal is to have the largest number with longest green bar. This function is controlled by performing the fine focus on the microscope.
   c. **Direction:** Keep this at "0". This is an electronic way to flip or rotate live image.
   d. **White balance:** You may use either point or rectangle area. With live image in focus, click on the "dropper" button, then click pointer on a white area on the live image window. Make sure that at the bottom of the tabs, the number is either 100 or 150 and on the microscope on the right side of the body, the light slider is in the middle at "camera" icon. Then click the start button, next to the dropper button and wait for the software to finish white balance.

4. **Timer:** There are several functions for time lapse imaging, which is not available on this microscope.

5. **Option:** It has five functions:
   a. **Image:** Select between color, black and white or negative
   b. **Enhancement:** Not recommended
   c. **Shading:** Not recommended
   d. **Noise reduction:** Not recommended
   e. **Auto print:** Not recommended

Below the tabs, there are three important functions:

1. **Sensitivity:** Use normal for routine powerpoint use, and high or max for publications.
2. **Light quantity:** This is displayed as numbers. This can be changed by sliding the bar under the number window. The lower the number, the brighter is the image. For 4x and 10x objectives, use numbers 150 and above. For 20x and 40x objectives, use 100, 80, or 50.
3. **Exposure:** Once you are happy with the live image, adjusted by manipulating appropriate functions listed above, click on the green exposure button, and the camera with acquire and automatically save the image in your selected folder.