PNWU
Virtual Microscope

Pacific Northwest University of Health Sciences

User's Manual for the Virtual Microscope Viewer

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I. **Introduction.** The PNWU Virtual Microscope consists of a database of "virtual slides" (digitally-scanned microscope slides) and computer software that allows the user to view these virtual slides. This virtual microscope is intended for use during the laboratory component of medical-school or health-science histology ("microscopic anatomy") courses.

One of the significant features of the PNWU Virtual Microscope is that the slide database includes high-resolution, multifocal-plane virtual slides (you can focus up-and-down through the specimen with multifocal-plane slides).

A. **The PNWU Virtual Microscope and its Viewer.** There are two software modules that form the core of the PNWU Virtual Microscope. The software that displays the list of slides is the "slide box" ("**PNWU Slide Box**", http://slidebox.pnwu.edu); the Slide Box is *not* the focus of this user manual (instructions for using Slide Box can be found on Slide Box's "About" menu). Once a slide has been chosen (see II.C. (below)), the software that displays the virtual slide is the "Viewer" (http://viewer.pnwu.edu). It is the Viewer that is described in this instruction manual.

B. **Talking to us.** Feedback from users is essential to improving the PNWU Virtual Microscope, and we welcome any constructive feedback that you may have. General comments regarding the Virtual Microscope can be emailed to: Microscope@PNWU.edu.

"To err is human"[1] and while we have tried eliminate as many computer-bugs as possible, there undoubtedly are a lot of bugs that we have not yet eliminated. However, the bugs can't be eliminated unless we know that they exist. If you encounter a computer-bug or other technical problem involving the PNWU Virtual Microscope, *please* let us know, preferably by emailing us at: MicroscopeBugs@PNWU.edu. If possible, please include a description of what the Viewer was doing when it encountered the computer bug *and* the text of the error-message that the viewer generated, assuming that viewer sent you an error message (the contents of the error message usually include information that helps us identify where the bug is hiding).

If you need to contact us and email won't work, our mailing address is:

PNWU Virtual Microscope
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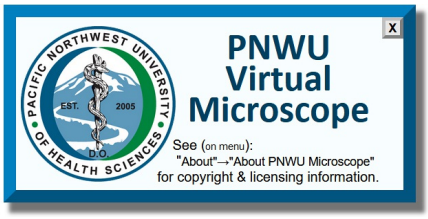
II. **Getting started ("How to: the basics").**

A. **Invoking the Viewer.** The PNWU Virtual Microscope is an HTML-based program that runs within an internet browser. The URL for this program is: "http://viewer.pnwu.edu". To run this program, type: viewer.pnwu.edu into the appropriate box of your internet browser and press <ENTER> (or <GO>, depending on the browser).

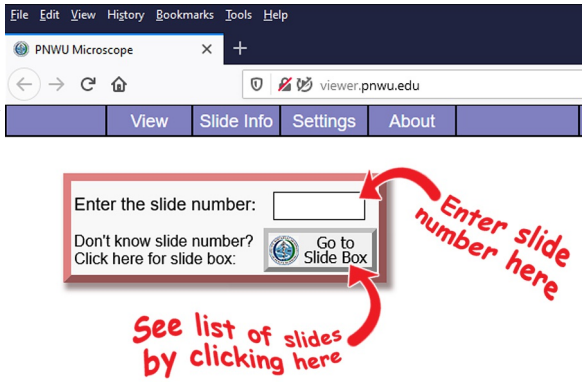
[1] Pope A. (1711) Essay on Criticism, line 526.

B. **"Logo" box.** While the Viewer is loading, a box transiently appears that displays the program title and refers the user to the "**About**" menu → "**About PNWU Microscope**" for copyright and licensing information (see VI.A. (page 33, below)).



Normally, the "Logo" box will fade away in about ~2½ seconds. However, in order to give the user more time to read the box's contents, moving the mouse cursor over this box (or touching the box for a touchscreen device) prevents the box from fading (until the mouse cursor is moved off the box). As a result, if the mouse cursor accidentally is in the same location as the "Logo" box when the Viewer loads, ***the "Logo" box will not fade*** (and will be in-the-way) ***until the user*** realizes the nature of the problem and ***moves the mouse cursor.***

C. **Choosing a slide.** After you have invoked the Viewer (see II.A. (above)), and the Viewer has finished loading, a box appears in the upper-left quadrant of the Viewer's window that asks you to "**Enter the slide number**". Each "virtual slide" in the database is identified by a unique slide-number (see II.E.1. (page 9, below)). If you know the number of the slide you wish to view, enter it into the text-box and press the <ENTER> key.

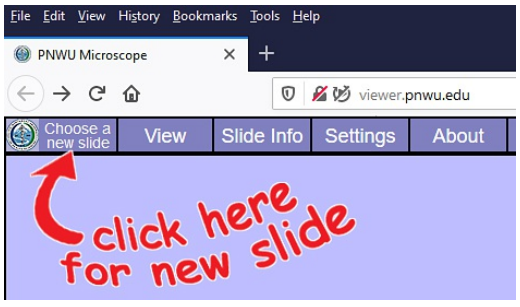


If you don't know the slide's number, clicking on the "**Go to Slide Box**" button will take you to a webpage (the "PNWU Slide Box": http://slidebox.pnwu.edu) that allows you to search PNWU's database of virtual slides.

Normally, clicking on the "Go to Slide Box" button causes the Slide Box to *replace* the Viewer within the internet browser. However, if the <SHIFT> key is depressed when the "Go to Slide Box" button is clicked, the Slide Box will open in a new tab or window (depending on your browser's settings) within the internet browser. If the <CNTRL> key (instead of nothing or the <SHIFT> key) is depressed when the "Go to Slide Box" button is clicked, then the Slide Box opens in the same tab of the internet browser that had displayed the Viewer, but the Viewer remains in the browser's "history" (so clicking the browser's "back" button takes you back to the Viewer).

If you know the slide number, you can skip a step by including "?slide=value" at the end of the URL when you invoke the Viewer (see II.A. (above)), where "value" is the number of the slide (e.g., entering: http://viewer.pnwu.edu?slide=3002 would cause the viewer to open with slide #3002 loaded; see II.F.1. (page 14, below)) for more about this option. Including the slide number in the URL suppresses display of the "**Choose a new slide**" button on the menu (see below); in this case, if you wanted to view another slide, you would need to re-invoke the Viewer (see II.A. (above)).

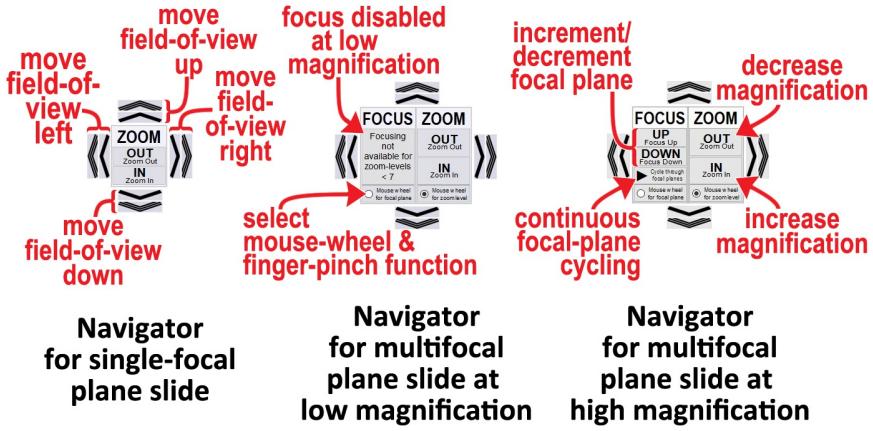
After you have chosen a slide and the slide is displayed by the viewer, the "**Enter the slide number**" box no longer is displayed. When you are finished examining the current virtual slide, you can choose another virtual slide by clicking on the "**Choose a new slide**" tab on the main menu. This will close the current slide and re-display the "**Enter the slide number**" box.



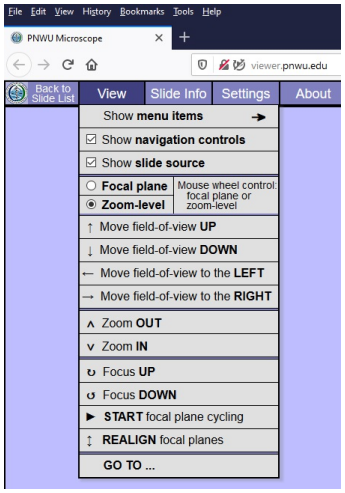
D. **Navigating around the slide.** After the "virtual slide" has been loaded into the viewer, you will want to examine various parts of the slide at varying magnifications, and (for multifocal-plane slides) at different focus levels. Thus, you need to be able to "move" the slide in the x-direction (left/right) & y-direction (up/down), to zoom in/out (magnification increases/decreases), and to focus up/down.

1. **Navigation controls.** There are several sets of controls that you can use to navigate around the slide:

   a. **Navigator.** After a slide has been chosen (and the slide appears on the screen), the navigator is displayed in the lower-right quadrant of the screen (it can be hidden using the "**View**" menu → "Show **navigation controls**" checkbox). For single-focal-plane slides, the navigator contains only the controls for "moving" the slide and for changing the magnification. For multifocal-plane slides, the navigator also has controls for changing the focus; however, these focus controls are only available when viewing the slide at high magnification.



   b. **Menu.** The same controls found on the navigator (see II.D.1.a, (above)) are also present on the "**View**" drop-down menu (except for the large-step ("triple-arrow") move buttons; see II.D.2.c (page 5, below)). To see the controls in this menu, position the mouse cursor over the tab labeled "**View**" on the menu (for touchscreen devices, touch the tab labeled "**View**").



   c. **Mouse.** The coordinates of the mouse cursor normally are displayed on the menu, and can be used to determine the location of features within the specimen (these coordinates are relative to the upper-left corner of the slide and are in pixels at the highest magnification; see III.A.4 (page 17, below)). Depressing the mouse's left button allows you to "move" the "virtual slide" by dragging it with the mouse (see II.D.2.a. (below)). The mouse's wheel can be used to change the magnification (see II.D.3.a. (page 6, below)) or to focus up/down (see II.D.4.c. (page 9, below)).

d. **Touchscreen control.** For touchscreen devices, touching the screen is an alternative to using the computer's mouse. Touching the "virtual slide" with ***one*** finger causes the location of the finger (relative to the upper left corner of the slide in pixels at the highest magnification) to be displayed on the menu. Touching the slide with ***two*** fingers allows you to "move" ("drag") the "virtual slide" (moving the two fingers in unison; see II.D.2.b. (below)) or to change magnification or focal plane (pinching/spreading the two fingers; see II.D.3.b. (page 6, below) & II.D.4.d. (page 9, below)).

2. **"Moving" the slide.** Except at very low magnifications, only a small part of the specimen will fit on the computer's screen. As a result, it is necessary to "move" the virtual slide in order to view different regions of the specimen. There are several ways that you can change the region of the specimen that is displayed on the computer screen.

   a. **Using the mouse to move the slide.** To drag the virtual slide using the computer's mouse, position the mouse cursor anywhere on the specimen, and depress the mouse's left button; the icon for the mouse cursor will change from the 'default' cursor icon (↖) to the 'move' cursor icon (✥). With the mouse's left button depressed, moving the mouse will move the specimen (and the mouse cursor) across the computer screen. Releasing the mouse's left button will disengage the virtual slide from the mouse cursor and restore the 'default' cursor icon.

      The mouse also can be used to move a structure to the center of the screen: depress the «CTRL» key (on the computer's keyboard) and then (while the «CTRL» key is still depressed) double-click the mouse's left button while the mouse cursor is positioned over on the part of the specimen that you want to center. The slide will "move" so that the object on which you double-clicked will be in the center of the screen.

   b. **Moving the slide with a touchscreen.** For a touchscreen device, the specimen can be "moved" by placing ***two*** fingers on the region of the computer screen displaying the specimen, and then moving these two fingers in unison across the computer screen. The two fingers will "drag" the specimen across the screen.

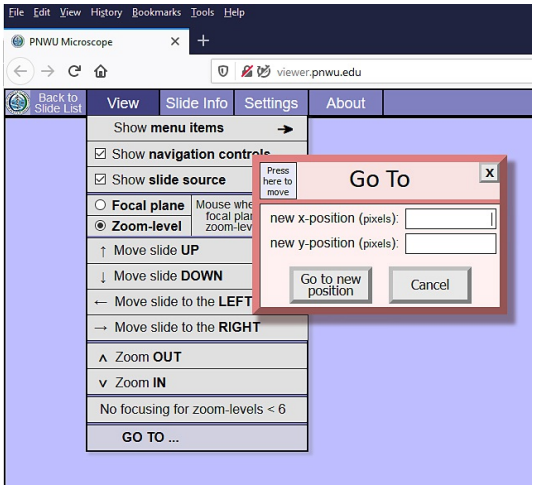
   c. **Using the navigator or menu controls to move the slide.** Both the navigator (see II.D.1.a, (above)) and the "**View**" menu (see II.D.1.b, (above)) have UP, DOWN, LEFT, and RIGHT controls ("buttons"). Depressing the mouse's button when the mouse cursor is positioned over one of these controls, or touching the UP/DOWN/LEFT/RIGHT controls with your finger (for a touchscreen device) causes the specimen to repetitively "step" (i.e., "move" in increments (e.g., ~20 screen-pixels)) across the screen until the mouse button is released or your finger is lifted off of the computer screen.

      Normally, activating one of these 'arrow' buttons (the UP, DOWN, LEFT, or RIGHT controls ("buttons")) causes the *field-of-view* to "move" in the direction indicated by the arrow, allowing you to see more of the specimen in the direction of the arrow. Many users of the PNWU Virtual Microscope want the navigator & menu 'arrows' to move the *field-of-view* in the direction indicated by the arrow (the default behavior described above). However, there are a significant number of users who want the 'arrow' buttons to have the opposite action, so that depressing the 'arrow' would cause the *slide* (rather than the *field-of-view*) to "move" in the direction indicated by the arrow. You can use the "**Settings**" menu → "Make arrows **MOVE SLIDE / FOV**" button to reverse the direction in which the navigator and menu 'arrow' controls move the specimen (see V.B.2. (page 20, below)).

The navigator has two sets of UP, DOWN, LEFT, and RIGHT controls (arrow "buttons"; see image on page 3 (above)). Activating one of the inner "single-arrow" buttons (by holding the mouse button down when the mouse cursor is over the navigator control, or by touching the control with your finger (for a touchscreen device)) results in the specimen moving in smaller steps (usually 20 screen-pixels/step), while activating one of the navigator's outer "triple-arrow" buttons results in the specimen moving in larger steps (usually 200 screen-pixels/step). The step-size for the "**View**" menu's UP, DOWN, LEFT, and RIGHT buttons is the same as for the inner ("single-arrow") buttons on the navigator. If the «SHIFT» key (on the computer's keyboard) is depressed when the menu or navigator's UP/DOWN/LEFT/RIGHT control is activated, the step-size is increased (usually by a factor of 10).

The default step-size is 20 screen-pixels for the navigator small-step ("single-arrow") and menu UP, DOWN, LEFT, & RIGHT controls, and is 200 screen-pixels for the navigator big-step ("triple-arrow") controls, and the default step-size multiplier (invoked if the «SHIFT» key is depressed) is a factor of 10. However, the viewer automatically decreases these values for low-magnification zoom-levels if the step-size would be more than ~10% of the specimen's size (in screen-pixels). The step-size, the step-size multiplier, and the time interval between repetitive steps (while the UP/DOWN/LEFT/RIGHT button is held down) can be changed using the "**Settings**" menu → "**Change settings ...**" control (see V.C.5 ("**Changing Settings**"; page 33, below)).

d. **"Go to ..."**. If you want to view a specific site within the specimen, and if you know the coordinates of that location, you can use the "**Go to ...**" control located on the "**View**" drop-down menu to "move" directly to that location.



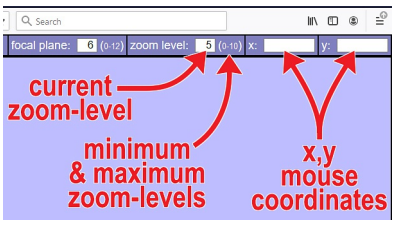
To use the "**Go to ...**" control, open the "**View**" drop-down menu by moving the mouse cursor over the "**View**" tab on the main menu (or by touching the "**View**" tab on a touchscreen device) on the main menu. Then click on the "**Go to ...**" control;[2] the "**Go to ...**" control is located at the bottom of the "**View**" drop-down menu.

When you click on the "**Go to ...**" button, a new box opens. Enter the coordinates of the location you wish to view in the appropriate spaces in this box, and then click the "**Go to new position**" button. The slide will "move" so the location whose coordinates were entered into "Go To" box will now be located in the center of the screen.

3. **Changing magnification.** Each virtual slide consists of sets of images at different magnifications, or zoom-levels (see II.E.7.b. (page 12, below)). The lowest magnification is zoom-level = 0, and each zoom-level has twice the magnification of the previous zoom-level. The number of zoom-levels varies from slide-to-slide, depending on the dimensions of the "real" specimen and the magnification at which this specimen was digitally scanned.

[2] When using the computer mouse, "clicking on" a control means depressing ***and*** releasing the mouse button while the mouse cursor is positioned over the control. For a touch-screen device, "clicking on" a control means touching the control ***and*** lifting your finger off the control. For a 'click', the control is activated *after* the 'up-and-down' action of the mouse button or finger has been *completed*.

The current zoom-level (and the minimum & maximum zoom-levels) normally is shown in an information box on the main menu.



Analogous to what was available for "moving" the specimen across the computer screen (see II.D.2. (page 4, above)), there are several controls that you can use to zoom-in (i.e., increase the magnification at which the specimen is viewed) and zoom-out (i.e., decrease the magnification).

a. **Using the mouse-wheel to change zoom-level.** For single-focal-plane slides (see II.D.4. (page 7, below) for more about focal planes), rotating the mouse-wheel changes the zoom-level.

   For multifocal-plane slides, the rotating the mouse-wheel changes *either* the focus (see II.D.4.c. (page 9, below)) *or* the zoom-level, depending on which of the "Mouse-wheel control" radio buttons (on the navigator (see II.D.1.a. (page 3, above)) and on the "**View**" drop-down menu (see II.D.1.b. (page 3, above)) has been selected. To set the mouse-wheel so that it controls zoom-level (for multifocal-plane slides), click on the "Mouse wheel for zoom-level" radio-button on the navigator or on the "**View**" drop-down menu (for "clicking on" a control, see footnote 2 (page 5, above)).

   If the mouse-wheel is set to control the zoom-level (see above), rotating the mouse-wheel towards the user zooms-out (decreases magnification), while rotating the mouse in the opposite direction (towards the computer screen) zooms-in (increases magnification).

   If you spin the mouse-wheel quickly, the viewer may temporarily enter a "wait state" before changing zoom-levels. "Wait states" are described in the section entitled **"Using 'Cycle through focal planes'"** (see II.D.4.b. (page 8, below)).

b. **Using a touchscreen to change zoom-level.** For a touchscreen device, *two fingers* touching the part of the screen displaying the specimen can be used to move the specimen, to change the zoom-level, or to change the focal plane (for multifocal-plane virtual slides). Moving two fingers in unison across the screen will drag the specimen across the screen (see II.D.2.b. (page 4, above)); pinching or spreading your fingers acts in the manner as rolling the mouse-wheel (see II.D.3.a. (above)) to change magnification or focal plane.

   As described for the mouse-wheel (see II.D.3.a. (above)), whenever the zoom-level is being changed, the viewer can enter a "wait-state" if the new zoom-level is missing image tiles; when using a touch-screen device, pinching/spreading two fingers on the specimen can cause the viewer to temporarily enter a "wait-state" (particularly if the server is heavily loaded or if you have a slow internet connection). "Wait states" are described in the section entitled **"Using 'Cycle through focal planes'"** (see II.D.4.b. (page 8, below)).

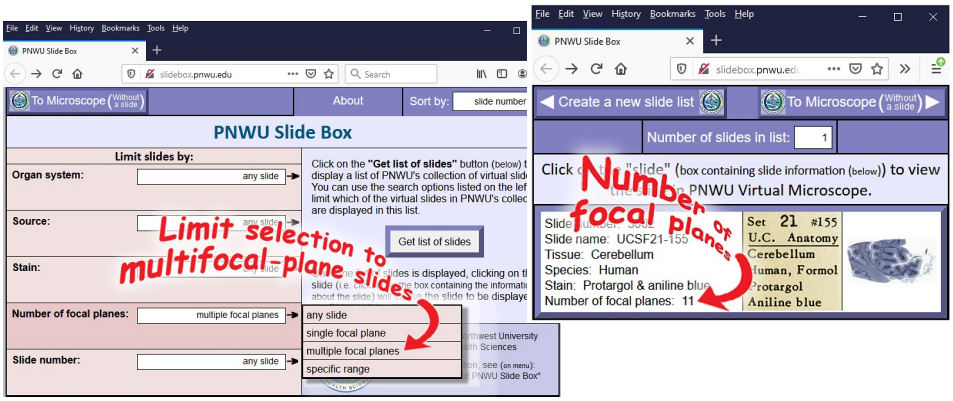
   Because of a computer-bug that we have not yet been able to exterminate, pinching or spreading your fingers usually will only change one or two zoom levels and then doesn't do anything more until you lift *both* fingers off of the screen. After lifting your fingers, you can then put your fingers back on the screen to change the zoom-level with another pinch or spread.

   For single-focal-plane virtual slides, pinching your fingers together (when both fingers are touching the part of the touchscreen displaying the slide) causes the magnification to decrease (zoom-out). Conversely, spreading your fingers (while they are touching the specimen displayed on a touchscreen device) causes the magnification to increase (zoom-in).

For multifocal-plane virtual slides, pinching or spreading your fingers will change *either* the zoom-level *or* the focal plane, depending on which "Mouse-wheel control" button on the navigator (or on the "**View**" drop-down menu) has been selected. To have pinching/spreading your fingers control magnification of multifocal-plane slides, use your finger to 'click' the "Mouse wheel for zoom-level" button on the navigator or on the "**View**" drop-down menu (pinching/spreading fingers is the same as rolling the mouse-wheel (see II.D.3.a. (above)); to 'click' a button on a touchscreen device, touch the button with your finger ***and*** then lift your finger off the touchscreen; see footnote 2 (page 5, above)). When the "Mouse wheel for zoom-level" button is selected, pinching your fingers together (when your fingers are touching the part of the touchscreen displaying the slide) causes the magnification to decrease (zoom-out). Conversely, spreading your fingers (while they are touching the specimen displayed on a touchscreen device) causes the magnification to increase (zooms-in).

c. **Using the navigator or "View" menu to change magnification.** To decrease the virtual slide's magnification click on the "Zoom OUT" button on the navigator (see II.D.1.a. (page 3, above) for explanation of the navigator's controls) or on the "**View**" drop-down menu (for "**View**" menu controls, see II.D.1.b (page 3, above); for "clicking on" a control, see footnote 2 (page 5, above)). Conversely, to increase the magnification, click on the "Zoom IN" button on the navigator or on the "**View**" drop-down menu.

4. **Changing focus.** In order to be able to focus up-and-down through a virtual slide, the virtual slide must include more than one focal plane. Thus, the focus can be changed *only* if the virtual slide being viewed is a multifocal-plane slide. Only *some* of the virtual slides in the PNWU database are multifocal-plane slides. If you are using the PNWU Slide Box to select the slide you wish to view (see II.C. ("**Choosing a slide**", page 2, above)), you can limit the list of slides to only multifocal-plane slides; the number of focal planes is included in the information about the slide listed in PNWU Slide Box's results.



The controls for changing focus are ***not*** displayed when viewing a single-focal-plane virtual slide. The controls for changing focus also are not available if the "Disable focusing" button on the "**Settings**" drop-down menu has been clicked (if focusing has been disabled, it can be restored by clicking on the "Enable focusing" button on the "**Settings**" drop-down menu; see V.A.4. (page 19, below)).

The ability to focus up-and-down requires considerable computer resources and can decrease the responsiveness of the Viewer to the user's actions. Since changes in focus usually are not apparent at low magnification, the PNWU Virtual Microscope is set so that

the ability to change focal planes usually is only available when a (multifocal-plane) slide is being viewed at its four highest zoom-levels. The minimum zoom-level at which focusing is allowed can be changed using the "**Settings**" menu → "**Change focus settings ...**" control (see V.A.3.b. ("**Change 'zoom-level limit for focus'**"; page 19, below)).

The total number of focal planes and the spacing between focal planes varies between virtual slides (these are determined when the "real" microscope slide is scanned to create the virtual slide). The focal planes are numbered from 0 to $n-1$, where "n" is the total number of focal planes available for that specific virtual slide. Normally, for multifocal-plane virtual slides, the current focal plane (and the minimum and maximum focal plane) are displayed in a box on the main menu (the box displaying this information is ***not*** included on the menu for a single-focal-plane slide). The distance (within the original specimen) separating adjacent focal planes is shown in the "**Slide Info**" drop-down menu (see II.E. (page 9, below); the current, minimum, and maximum focal-plane numbers also are shown in the "**Slide Info**" drop-down menu).

a. **Using "Focus UP" & "Focus DOWN" buttons to change focus.** For multifocal-plane virtual slides, both the navigator (see II.D.1.a. (page 3, above)) and the "**View**" drop-down menu (see II.D.1.b. (page 3, above)) will display controls ("buttons") labeled "Focus **UP**" and "Focus **DOWN**" *if the zoom-level is equal to or greater than the zoom-level limit for focusing*. Clicking on the "Focus **UP**" button increments (by one) the number of the focal plane that is being displayed; clicking on the "Focus **DOWN**" button decrements the number of the focal plane being displayed (for "clicking on" a "button", see footnote 2 (page 5, above)).

b. **Using "Cycle through focal planes".** There are many instances when the user will want to repetitively focus up-and-down through the entire stack of focal planes (in order to get a sense of the three-dimensional structure of a cell or region). Clicking on the "**Cycle through focal planes**" button on the navigator or "**Start focal plane cycling**" on the "**View**" drop-down menu will cause the viewer to step-wise increase the focal planes (up to the maximum focal plane), then step-wise decrease the focal plane (down to focal plane = 0), and then repeat the process until the user clicks on the "**Stop focal plane cycling**" button, or the user initiates a different action. Moving the specimen (see II.D.2. (page 4, above)), or changing zoom-level (see II.D.3. (page 5, above)) automatically stops focal plane cycling. As is the case for all focus-controls, the "**Cycle through focal planes**" button is only available if the magnification (zoom-level) is greater than the zoom-level-limit for focusing.

When cycling through focal planes, the viewer normally pauses for about ¼ second at each focal plane. You can increase or decrease the rate at which the viewer cycles through the focal planes using the "**Settings**" menu → "**Change focus settings ...**" control (see V.A.3. ("**Focusing controls**"; page 19, below)).

Wait-state. It is not uncommon for the viewer to repetitively enter a "wait-state" during the first cycle of focusing up-and-down after the user clicks on "**Cycle through focal planes**" button. Cycling through focal planes should proceed smoothly after the first cycle of focusing up-and-down; these wait-states should occur only during the first cycle.

A "wait-state" occurs when the viewer attempts to change view-planes before all of the image-tiles for the new view-plane have been loaded (view-planes are explained in footnote 11 (page 21, below)); in this situation, the viewer pauses (in a wait-state) until the missing image-tiles have been loaded (see V.C.3.c. (page 31, below); during the 1st cycle of "cycling through focal planes", the cycling rate often outruns the ability of the Viewer to pre-load the focal planes). During a wait-state, the icon for the mouse cursor will change to a "wait cursor" icon (usually an hourglass: ⌛ or spinning wheel: ○) and a touchscreen device will display a "wait icon" (a spinning clock face: ◷) if two fingers are touching the touchscreen. The Viewer will not respond to commands to move the slide (see II.D.2. (page 4, above)), change the zoom-level (see II.D.3. (page 5, above)), or change the focal plane while in a wait-state.

c. **Using the mouse-wheel to change focus.** For multifocal-plane slides, rotating the mouse-wheel either changes the zoom-level (see II.D.3.a. (page 6, above)) or the focus, depending on which "Mouse-wheel control" radio-button has been selected (on the navigator (see II.D.1.a. (page 3, above)) and on the "**View**" drop-down menu (see II.D.1.b. (page 3, above)). To set the mouse-wheel so that it changes the focal plane (for multifocal-plane slides), click on the "Mouse wheel for focal plane" radio-button on the navigator or on the "**View**" drop-down menu (for "clicking on" a control, see footnote 2 (page 5, above)).

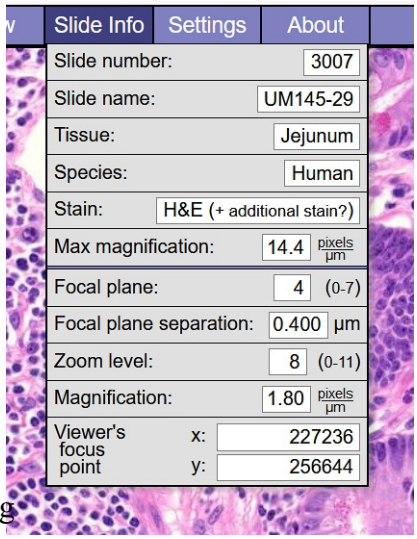
If the mouse-wheel is set to control the focal plane (see paragraph above), and if the magnification (zoom-level) is greater than the zoom-level limit for focusing (see II.D.4. (page 7, above)), rotating the mouse-wheel towards the user will focus **up** (increasing the focal-plane number), while rotating the mouse in the opposite direction (towards the computer screen) focuses **down** (decreasing focal plane number).

If you spin the mouse-wheel quickly, the Viewer may temporarily enter a "wait-state" before changing focal planes. Wait-states are described in the section entitled **"Using 'Cycle through focal planes'"** (see II.D.4.b. (above)).

d. **Using the touchscreen to change focus.** For a multifocal-plane virtual slide viewed on a touchscreen device, with two fingers touching the image of the specimen, pinching your fingers together or spreading them apart to will focus up or down (respectively) ***if*** the "Mouse-wheel for focal plane" radio-button (on the navigator or on the "**View**" drop-down menu) has been selected (see discussion ("**Using a touchscreen to change zoom-level ... For multifocal-plane virtual slides**") in II.D.3.b. (page 7, above) for using pinching/spreading fingers to control either magnification or focus) and if the magnification (zoom-level) is greater than the zoom-level limit for focusing.

E. **Information about the slide.** Much of the information about the virtual slide is contained in the "**Slide Info**" menu. This information will appear in a drop-down menu when the computer's mouse cursor is over the tab labeled "**Slide Info**" on the main menu (or when you touch this tab with your finger (for a touch-screen device)). In addition, some of this information usually is more readily accessible on the Viewer's main menu.

1. **Slide number.** Every virtual slide in the PNWU database is assigned a unique number (a positive integer) which is used by the PNWU Virtual Microscope to identify the slide. You can include this number (which is displayed on the "**Slide Info**" menu) in the Virtual Microscope's URL (see II.C. (page 2, above)) to directly access the slide; this can be helpful if you want to discuss a specific slide with a colleague (for including commands in the URL, see II.F (page 13, below)).



2. **Slide name.** In addition to the slide number (see II.E.1. (above)), each virtual slide also has a name assigned to it. Although frequently cryptic (to keep the name short), this name often identifies the specific glass microscope slide that was scanned (e.g., the slide-box number and specimen number) or identifies the virtual slide in a shared database, and this name may be easier to remember than the slide number. In addition to always being displayed on the "**View**" drop-down menu, this name usually is shown on the main menu (display of the slide name on the main menu can be toggled on/off using the "**View**" menu → "Show **menu items**" → "Show **slide name** on menu" checkbox (see III.A.1. (page 17, below))).

3. **Tissue.** A short description identifying the organ(s) or tissue(s) present on the slide is listed on the "**Slide Info**" menu. This description is intended to help when identifying the slide from within a list of slides; it is ***not*** intended to be a complete listing of all of the structures present on the slide.

   For instance, slide UM130-75 (Tissue: "Seminal vesicle, etc.") includes sections of the seminal vesicle, but it also contains a cross-section of the ampulla of the ductus deferens, part of the prostate gland, and an autonomic ganglion (in addition to blood vessels, nerves, adipose tissue, loose connective tissue, and the cells & basic tissues that comprise the principal organs). Similarly, slide UCSF172-485 ("Brain, pituitary, etc."), in addition to brain (cerebellum, midbrain, & diencephalon, and meninges) and pituitary gland, also contains bone, bone marrow, cartilage, skeletal muscle, and nasopharynx.

   In many cases, information contained in the slide label was used when deducing the tissue or organ of origin. The information included in the slide label may not be entirely accurate, and as a result, the information listed in the "tissue" box in the "**Slide Info**" menu also may not be entirely accurate.

4. **Species.** The trivial name for the species (e.g., "human" for *Homo sapiens*, "mouse" for *Mus musculus*, or "monkey" for any of several "lower" primate species) from which the tissue was obtained is listed in the "**Slide Info**" menu when this information is available. This information (which usually is obtained from the slide label or other information associated with the specimen) may not be accurate, and in some cases a "?" has been appended to the name of the organism when there are reasons to doubt the species assignment.[3]

5. **Stain.** The stain used to add contrast to the specimen is listed in the "**Slide Info**" menu (for space reasons, the name of the stain may be abbreviated; see XI. (page 49, below)). This information usually is obtained from the slide label (or other information associated with the specimen) or is deduced from the appearance of the specimen, and it may not be entirely accurate. In cases where there are reasons to doubt the stain listed (e.g., because the appearance of specimen suggests a stain that is different from that listed on the slide label), a "?" may follow the name of the stain.

[3] For instance, the species for UCSFx-027 is listed in the "Slide Info" menu as "Monkey?". The label attached to this slide identifies the tissue as "human"; however, the capsule and trabecula of human spleen (unlike those of many other mammals) usually does *not* contain smooth muscle, and smooth muscle is present in the capsule (see UCSFx-027(2D)) and trabecula of this specimen. The label for another slide from the same block of tissue (UCSF-27) indicates that the specimen is from a "monkey", which (on the basis of the histology) is more likely than "human". Given the uncertainty surrounding the provenance of this specimen, "?" is warranted .

6. **Ownership & licensing.** The scanned image of a microscope slide might be considered a "creative work" (similar to a photograph or work of art) and, therefore, the virtual slides displayed by the PNWU Virtual Microscope may be protected by copyright. The person and institution that provided the scanned slide (the presumptive copyright holder) is shown in the upper-right corner of the specimen window (this information can be toggled on/off using the "**View**" menu → "Show **slide source**" checkbox; see III.B. (page 17, below)). Although they may be protected by copyright, most of the scanned slides in the PNWU virtual-slide database are provided under a Creative Commons license. If available, the Creative Commons license is listed, in addition to the information about the person and institution who provided the slide, in the upper-right corner of the specimen window.

7. **Location within the specimen.** It often is useful to know your location within the specimen, particularly if you find a structure that you might want to study again at a later date. This information is available on the main menu or within the "**Slide Info**" menu.

   a. **X,Y coordinates.** The x- and y-coordinates within the specimen of the computer's mouse cursor, or of a single finger touching the screen (for a touch-screen device), usually are listed on the Viewer's main menu (on the top of the Viewer's window). Display of this information can be toggled on/off using the "**View**" menu → "Show **menu items**" → "Show **x & y position** on menu" checkbox (see III.A.4. (page 17, below)).

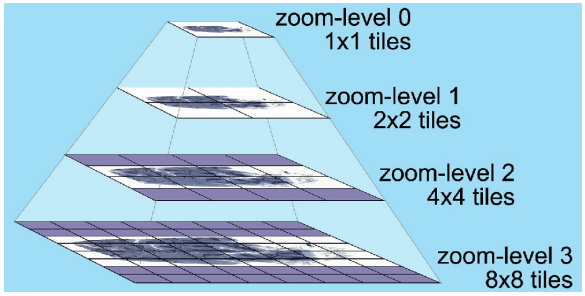
   These coordinates are the distance in specimen-pixels (pixels at the highest magnification, which is the magnification at which the specimen was scanned) from the top-left corner of the specimen. A location within the specimen has the same x,y-coordinates regardless of zoom-level[4] or focal plane. You can use these coordinates to determine your current location when moving the specimen across the viewer's window. If you note the coordinates of a particular location within the specimen, these coordinates can be used later in the "Go to ..." box (see II.D.2.d (page 5, above)) to return to this particular location. These coordinates also can be included in command-line arguments (see II.F (page 13, below); "x=value" and "y=value") to cause the slide to open with the specimen centered on these coordinates.

   The coordinates of two points also can be used to calculate the distance (in the original specimen) between the two points, which can be used to determine the sizes of objects (e.g., cells, nuclei, ducts or blood vessel diameters, *etc.*) within the specimen (see II.E.7.b. ("**Zoom level & magnification**", below))

   Unlike the other data listed in information boxes on the main menu (see II.E.2. (above), II.E.7.b. (below), and II.E.7.c. (below)), the x- and y-coordinates of the mouse cursor (or single finger) are listed only in the information boxes on the main menu; these coordinates are ***not*** listed in "**Slide Info**" drop-down menu (the coordinates (on the specimen) of the mouse's cursor cannot be displayed on the "**Slide Info**" menu because the mouse cursor has to be moved off the specimen (and onto the menu) in order for the "**Slide Info**" menu to drop-down). Listed on the "**Slide Info**" menu are the x- & y-coordinates of the "Viewer's focus point"; most of the time, these x,y-coordinates are the coordinates (in specimen-pixels) of the center of the viewer's window.

[4] The x,y-coordinates at different zoom-levels will be affected by rounding errors. The computer determines the location of the mouse (or your finger) in screen-pixels (pixels at the current magnification), and since pixels are integers, the x,y-coordinates (in specimen-pixels) determined at low-magnification zoom-levels will be rounded (truncated) to the nearest screen-level pixel.

b. **Zoom level & magnification.** Each virtual slide consists of series of sets of images (image-tiles), with each set of images being at a different magnification and constituting a "zoom level". The number of zoom levels is dependent on the area of the "real" specimen (e.g., on a glass microscope slide) that was scanned and the magnification at which the "real" specimen was scanned (using a "real" microscope). For the PNWU Virtual Microscope, the highest zoom level is at the magnification at which the "real" slide was scanned, and each subsequent (lower) zoom level has a magnification that is ½ that of the previous magnification. The magnification at the lowest zoom-level (zoom level = 0) is such that the entire image fits on a single 256×256-pixel tile.



On main menu. Normally, the current zoom level is displayed on the Viewer's main menu (at the top of the Viewer's window; see II.D.3. ("Changing magnification", page 5, above)). Display of the zoom level on the main menu can be toggled on/off using the "**View**" menu → "Show **menu items**" → "Show **zoom-level** on menu" checkbox (see III.A.3. (page 17, below)).

On "Slide Info" menu. Even when it is not displayed on the main menu, the current zoom-level can be seen on the "**Slide Info**" drop-down menu. In addition, if it is known, the magnification of the image at the current zoom level ("Magnification"; in pixels/µm or pixels/mm) is displayed below the zoom level on the "**Slide Info**" menu.[5]

Knowing the magnification of the displayed image (i.e., the zoom-level-specific magnification) can be useful, for instance, if you grabbed a screen-shot and wanted to add a scale-bar to the image, or if you wanted to analyze the size/shape of a structure from a screen-shot that had been imported into image-analysis software.

Maximum magnification. If the magnification at which the "real" slide was scanned is known, the "**Slide Info**" menu shows the "Max magnification", which is the magnification (in pixels/µm) at the highest zoom level for that slide. Since the x,y-coordinates are given as pixels at this magnification (see II.E.7.a. (above)), the coodinates of two points in the specimen and "Max magnification" value can be used to calculate the absolute distance (in µm) between the two points.

For instance, if the coordinates of the first point are x_1,y_1 and the coordinates of the second point are x_2,y_2, then the actual distance separating the two points in the "real" specimen would be:

$$\begin{aligned}\text{distance } (\mu m) &= \text{distance (pixels)} \div \text{max.magnification (pixels/}\mu m) \\ &= \left(\sqrt{\left((x_2 - x_1)^2 + (y_2 - y_1)^2\right)}\right) \Big/ \text{max.magnification}\end{aligned}$$

[5] There are two magnifications listed on the "**Slide Info**" menu. The "max magnification" is the magnification of the image at the highest zoom-level; this also was magnification of the image (in pixels/µm) when the image was scanned. Both the "max magnification" and the x,y-coordinates listed in the main-menu are in reference to the original specimen's image-space; the pixels are "specimen-pixels". The other magnification (identified as "Magnification" on the "**Slide Info**" menu), is the magnification achieved on the user's computer screen; the "pixels" in this measurement are the pixels of the screen that is displaying the image (screen-pixels). If the user knows the size of the specimen on the computer screen (in screen-pixels), then the "magnification" listed in the "**Slide Info**" menu can be used in conjunction with specimen's size (in screen-pixels) to determine the original size (in actual µm) of the specimen. Similarly, if part of the screen is copied to an digital image (i.e., a "screen-grab"), the resulting image most likely is in screen-pixels.

For virtual slides obtained from external databases, the "maximum magnification" value usually was extracted from metadata that was stored with the slide in the external database, and this datum may not be accurate (particularly for slides that have had their formats converted multiple times as they passed through several databases).

c. **Focal plane & focal plane separation.** Unlike a "real" microscope, where the user can focus continuously through an infinite number of infinitesimally-separated optically-overlapping focal planes, virtual slides in the PNWU Virtual Microscope contain a finite number of focal planes that (in the original "real" specimen) were separated by discrete distances.[6] As discussed earlier (see II.D.4. (page 7, above)), the number of focal planes included in each virtual slide varies between virtual slides; within the virtual microscope, these discrete focal planes are numbered from 0 to $n-1$, where "n" is the number of focal planes.

If the virtual slide consists of only a single focal the information about focal planes is moot and is ***not*** displayed.[7]

If the virtual slide contains multiple focal planes, the number of the current focal-plane (between 0 to $n-1$, where "n" is the number of focal planes) usually is displayed on the **main menu** (at the top of the Viewer's window; display of this focal-plane number can be toggled on/off using the "**View**" menu → "Show **menu items**" → "Show **focal plane** on menu" checkbox (see III.A.2. (page 17, below))). In addition (if the virtual slide contains multiple focal planes), the "**Slide Info**" **menu** lists both the focal plane number and the distance (in the original specimen) that separates adjacent focal planes.

F. **"Command-line" arguments (the short-version).** The PNWU Virtual Microscope is designed so that instructions ("commands") can be given to the Viewer as part of the Viewer's URL (the "command-line"). Although there are many commands ("command-line arguments") that can be passed to the Viewer via the URL, only a few of these commands probably are (or should be[8]) of interest to most users. The command-line arguments that are most likely to be used are listed here; a complete listing of all command-line arguments are listed near the end of this document (see IX. (page 38, below)).

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[6] The number of, and separation between, focal planes in a virtual slide is determined on the basis of factors such as computer & microscope hardware limitations and the thickness of the original specimen when the original specimen (the "real" glass microscope slide) is scanned. Because the spacing between focal planes (often ≥ 400nm) usually is greater than the Nyquist limit for the microscope (the Nyquist limit for a high resolution optical microscope is about ~250nm, similar to its resolving power (Rayleigh limit)), structures (or parts of structures) between focal planes may be missing from the virtual slide (e.g., look closely at the continuity of some of the mitotic chromosomes on slide UM237-3). Focusing up-and-down through a virtual slide (rather than a "real" slide) is similar to watching a movie of a baseball game (rather than watching the "real" ball game); in the movie, rather than seeing the continuous motion of the bat swinging, we see infer the motion of the bat from a series of discrete movie 'frames' of the batter at various stages of hitting the ball. If the interval between movie frames is too large, the bat's motion appears 'jerky'.

[7] For a single-focal-plane virtual slide, the "Focal plane" is always "0" and "Focal plane separation" is infinite.

[8] Some of the command-line arguments were included to anticipate unusual or 'difficult' internet environments, and are ***not*** intended for general use. Most of the Viewer's command-line arguments probably are best avoided (notably, the "cs_command=value" commands) unless their use has been discussed with the network system administrator.

Within the URL, the first command-line argument should be separated from the principal part of the URL ("http://viewer.pnwu.edu") by a question mark ("?"). If multiple commands are included in the URL, each command (after the first command) should be preceded by an ampersand ("&"). The commands are insensitive to upper-case *vs.* lower-case and extra white-space is ignored.

1. **Loading a specific slide ("http://viewer.pnwu.edu?Slide = value").** The command-line argument: "Slide=value" causes the viewer to load a specific slide; "value" is the desired slide's 'slide number' (see II.E.1. (page 9, above)). The "slide=" part of this command can be omitted; a command consisting solely of a positive integer, "value", ("http://viewer.pnwu.edu?value") is interpreted by the Viewer as "Slide=value" ("http://viewer.pnwu.edu?Slide = value"). For instance, to load slide #3004 (UCSF35-215), the URL http://viewer.pnwu.edu?3004 is equivalent to the URL http://viewer.pnwu.edu?slide = 3004.

   The PNWU Virtual Microscope is intended to be used for teaching Histology labs where there is a handout (Lab-instruction Guide) that, after asking the student to load a specific slide, provides instructions and questions regarding this specific slide. In this case, rather than makng the student go through multiple steps to open the Viewer and then load the slide, this command allows the internet-link in the handout to automatically load the correct slide for the student (in addition to opening the Viewer). Within the lab-handout, the link can be an anchor embedded within the text. For instance, the text within the handout might read something like: "Next, look at slide #UM130-75, and find the ganglion containing the cell bodies of post-ganglionic autonomic neurons. ...", where the link attached to "#UM130-75" is "http://viewer.pnwu.edu?slide=3005" or "http://viewer.pnwu.edu?3005".

   Because this command is intended to be used in conjunction with a lab-handout that obviates the need for the user to work with (or even know about) the list of slides in the PNWU Virtual Slide Box, the "Slide=value" command-line argument also suppresses the "Choose a new slide" tab (on the main menu; see "Choosing a slide", II.C. (page 2, above)).

   Particularly when the "Slide=value" command is used in a lab-handout, the command "Hide Logo" also may be useful. As recommended by the US Copyright Office,[9] the PNWU Virtual Microscope normally transiently displays a reference to its copyright when the Viewer loads (see II.B. (page 2, above)). In the case of a Histology lab, where students will be repetitively calling the Virtual Microscope's Viewer, showing the copyright notice every time the student loads a new slide seems to be overkill, and waiting 2½ seconds for the notice to fade gets to be annoying after the third or fourth slide. Display of the copyright notice can be truncated by including "...& Hide Logo &..." in the command-line arguments in the URL (i.e., "http://viewer.pnwu.edu?Slide = value & HideLogo"; see IX.7. (page 39, below)). The copyright notice should be displayed for the first slide loaded from the lab-handout, but the "...& Hide Logo &..." command can be included in the remaining links from the lab handout to the Viewer. For instance: "First, study the prostate gland in slide #UM3012-281, noting the relationship of the fibromuscular connective tissue to the epithelium of the prostatic secretory acini. ... Next, look at slide #UM130-75, which contains sections of both seminal vesicle and prostate glands, and find the sections of prostate gland in this specimen. ...". In this case the link

[9] "*For works published in machine readable copies ... a notice may be acceptable if it appears in the following manner ... The notice is displayed at the user's terminal at sign-on*" (section 2207.9, page 21; Compendium of U.S. Copyright Office Practices (https://www.copyright.gov/comp3/, chapter 2200: https://www.copyright.gov/comp3/chap2200/ch2200-notice.pdf)).

for slide #UM3012-281 (http://viewer.pnwu.edu?1645) does not include the "Hide Logo" command (so the copyright notice is displayed), while the link for slide #UM130-75 (http://viewer.pnwu.edu?slide=3005&HideLogo) includes the the "Hide Logo" command (and display-time for the copyright notice is truncated).

2. **Going to a specific location ("http://viewer.pnwu.edu? X=value1 & Y=value2 & Z=value3 & F=value4 & Slide=slideNumber ").** Sometimes we want to share something we've found on a slide with colleagues. The PNWU Virtual Microscope can provide precise information about the location of point within the specimen, such as the location of a cell or other structure of interest (see: II.E.7. (page 11, above)), but sharing the location without command-line arguments can be awkward. For instance, we could send our colleague an email saying: "*To see a lymphocyte undergoing diapedesis, open slide* #UCSFx-027(3D), *then use the "*Go To*..." box on the "*View*" menu to go to coordinates:* x = 34100, y = 31170, *then use the zoom-level control on the Navigator to increment the zoom-level to* zoom-level=8, *and then use the focus control on the Navigator to increment the focus to* focal plane=8". Fortunately, we can combine all these steps into a series of command-line arguments, and just send our colleague an email saying: "*See the lymphocyte undergoing diapedesis in the center of the field (*x=34100, y=31170*) on slide* #UCSFx-027(3D)"; in this case, the URL embedded in "#UCSFx-027(3D)" would be: "http://viewer.pnwu.edu/?slide=3001&z=8&f=8&x=34100&y=31170". In this URL, the location information is included in four separate "command-line" arguments (with an "&" between each command).

   a. **"x =** value **".** This command moves the slide so that value is the x-coordinate (see II.E.7.a. (page11, above)) of the center of the viewer's window when the slide is initially displayed (this x-coordinate is in specimen-pixels (see footnote 5 (page 12, above)); i.e., it is equivalent to the value listed as the x-coordinate in the information box on the main menu).

   b. **"y =** value **".** This command moves the slide so that value is the y-coordinate (see II.E.7.a. (page11, above)) of the center of the viewer's window when the slide is initially displayed (this y-coordinate is in specimen-pixels (see footnote 5 (page 12, above)); i.e., it is equivalent to the value listed as the y-coordinate in the information box on the main menu).

   Although it is unlikely that someone would want to set the x-coordinate without also setting the y-coordinate, the commands that set the x- and y-coordinates ("x = valueX ", and "y = valueY ") are independent and could be used separately.

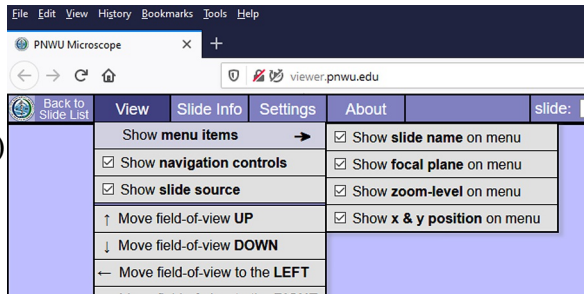
   c. **"z =** value **".** This command causes the zoom-level to be value (see II.E.7.b. (page12, above)) when the slide is initially displayed.

   d. **"f =** value **".** This command causes the focal-plane to be value (see II.E.7.c. (page 13, above)) when the slide is initially displayed.

   These location-determining command-line arguments ("x = valueX ", "y = valueY ", "z = valueZ ", and "f = valueF ") only affect the location within the specimen that is initially displayed. After the slide has finished loading, the user still can move around the slide using the navigation controls (see II.D. ("Navigating around the slide", page 3, above)).

III. **"View" menu.** Moving the computer mouse over the "**View**" tab on the main menu (or touching the main menu's "**View**" tab with your finger (for a touch-screen device)) causes the "**View**" drop-down menu to appear below the main menu's "**View**" tab. Most of the controls listed on the "**View**" drop-down menu have already been discussed (see II.D. ("Navigating around the slide", page 3, above)). However, there are two groups of controls (and a minor control) that have not yet been described.

A. **Controlling the main menu ("Show menu items ➜").** Positioning the mouse cursor over (or touching with your finger on a touch-screen computer) the tab labeled "Show **menu items ➜**" on the "**View**" drop-down menu causes a side-menu to appear to the right of the "**View**" drop-down menu. This side-menu contains check-box controls that allow the user to determine which information boxes appear on the main menu.



The main menu (located at the top of viewer's window) consists of a series of tabs (buttons) that activate drop-down menus, and boxes containing information about current slide (see II.D. ("Navigating around the slide", page 3, above) & II.E. ("Information about the slide", page 9, above)). The tabs for the drop-down menus are fixed, but the user can determine which of the information boxes are displayed on the main menu. This can be significant because the space in the main menu is limited.

If the width of the Viewer's window is sufficient,[10] the main menu (located at the top of Viewer's window) will consist of a single row of tabs & information boxes. If the width of the window is insufficient to fit the drop-down menu tabs and selected information boxes in a single row, the main menu will be folded into two rows, with the drop-down menu tabs in the upper row and the information boxes in the lower row. The user can restore the main menu to a single row by hiding some or all of the information boxes; this is done using the check-boxes in the "Show **menu items ➜**" side-menu (see below). With the exception of the x,y-coordinates (which require the mouse to be on the specimen rather than on a drop-down menu), the information shown in the boxes on the main menu also is available on the "**Slide Info**" drop-down menu (see II.E. ("Information about the slide", page 9, above)), so this information is still available even if the main-menu information boxes are hidden.

If the width of the Viewer's window is too small to fit all of the information boxes on the second line of a two-row menu, then the Viewer will automatically hide a sufficient number of information boxes so that the remaining boxes will fit in the second row of the menu. In this case, the user can use the the check-boxes in the "Show **menu items ➜**" side-menu to determine which information boxes remain visible (unless the user intervenes, the Viewer will hide the left-most information boxes).

[10] Currently (December, 2020), the minimum width to fit everything in the main menu onto one line is ~1240 pixels. If all of the information boxes except for the x,y-coordinates are hidden (see text above), the minimum width for a single-row menu is ~710 pixels. The height of each line (row) of the menu is 32 pixels.

There are four check-boxes in the "Show **menu items ➜**" side menu:

1. **"Show slide name on menu".** This check-box toggles whether the main-menu contains an information box for the slide name (for more about the slide-name, see II.E.2. (page 10, above)).
2. **"Show focal plane on menu".** This check-box toggles whether the main-menu contains an information box displaying the current focal plane (and the minimum & maximum focal planes; for more about focal-plane numbers, see II.E.7.c. (page 13, above)).
3. **"Show zoom-level on menu".** This check-box toggles whether the main-menu contains an information box displaying the current zoom-level (and the minimum & maximum zoom-levels; for more about zoom-levels, see II.E.7.b. (page 12, above)).
4. **"Show x & y position on menu".** This check-box toggles whether the main-menu contains the two information boxes that display the x- and- y coordinates of the mouse cursor (or, for a touch-screen device, a single finger touching the screen). Unlike the other main-menu information boxes, the x,y-coordinates are ***not*** displayed in the "**Slide Info**" drop-down menu. The units for the x,y-coordinates displayed on the main-menu are specimen-pixels (i.e. pixels at the highest magnification) from the top-left corner of the slide (for more information about the x & y coordinates, see II.E.7.a. (page 11, above)).

B. **Displaying items on slide-display window.** After the slide has been loaded, several other objects usually are displayed in the same window as the virtual slide (these are "on top of" the specimen). However, although these items usually should be visible (because they are useful or necessary), screen space is valuable and there are times (such as when grabbing an image of the specimen (taking a screen-shot), or when the viewer's window is very small) when the user might want to hide these objects in order to have an unobstructed view of the specimen. Display of these objects can be toggled on/off using checkboxes located on the "**View**" drop-down menu:

1. **"Show navigation controls".** This check-box toggles whether the navigator is displayed (for more information about the navigator, see II.D.1.a. (page 3, above)).
2. **"Show slide source".** This check-box toggles whether the source and license information about the slide (see II.E.6. (page 11, above)) is displayed.

C. **Navigation controls.** The "**View**" menu contains controls that duplicate the controls found on the navigator (see II.D.1.a, (above), except for the large-step ("triple-arrow") move buttons (see II.D.2.c. (page 5, above)). Instructions for using these "**View**"-menu controls to move the slide (see II.D.2.c. (page 4, above) & II.D.2.d. (page 5, above)), change magnification (see II.D.3.c. (page 7, above)), and change focus (see II.D.4.a. (page 8, above) & II.D.4.b. (page 8, above)) were provided earlier in this document.

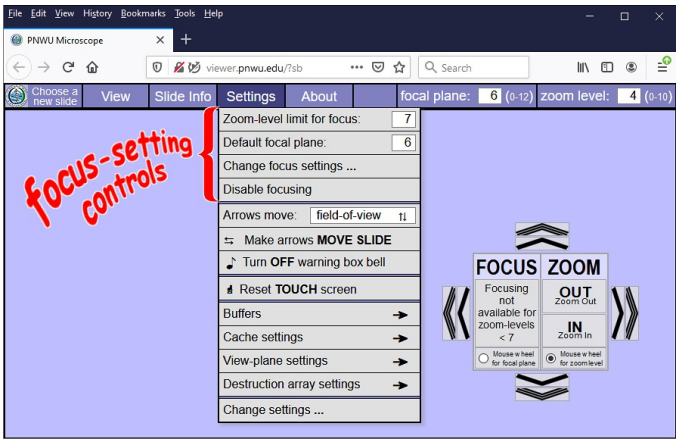
D. **Miscellaneous "View" menu item: "Realign** focal planes**".** You probably will never need to use the "Realign focal planes" control because the Viewer automatically aligns the focal planes before switching between focal planes. However, it is critical that the x,y-axes of the focal planes are aligned when focusing up-and-down through the specimen and if the focal-planes somehow are not aligned, clicking on this control will cause the Viewer to realign the focal planes. As is the case for all of the focusing controls on the "**View**" menu (see II.D.4. (page 7, above)), the "**Realign** focal planes" control is only displayed if the slide is a multifocal-plane specimen and if the magnification (zoom-level) is greater than the zoom-level limit for focusing.

IV. **"Slide Info" menu.** Moving the computer mouse over the "**Slide Info**" tab on the main menu (or touching the main menu's "**Slide Info**" tab with your finger (for a touch-screen device)) causes the "**Slide Info**" drop-down menu to appear below the main menu's "**Slide Info**" tab. The items in the "**Slide Info**" drop-down menu already have been described in the "**Getting Started**" section (see II.E. ("**Information about the slide**", page 9, above)).

V. **"Settings" menu.** Moving the computer mouse over the "**Settings**" tab on the main menu (or touching the main menu's "**Settings**" tab with your finger (for a touch-screen device)) causes the "**Settings**" drop-down menu to appear below the main menu's "**Settings**" tab.

The behavior of the PNWU Virtual Microscope is controlled by a number of variables ("settings") that are intrinsic to the javascript computer code that controls the webpage's response to the user's actions. The "**Settings**" menu displays the values of many of these variables, and provides the user with the ability to adjust these values.

A. **Focus settings.** The basics of focusing up-and-down through a multifocal-plane virtual slide were described earlier in this document (see II.D.4. ("**Changing Focus**", page 7, above)). As was the case for other focusing controls, the focus-related section of the "**Settings**" menu is displayed only if the slide being viewed is a multifocal-plane slide.



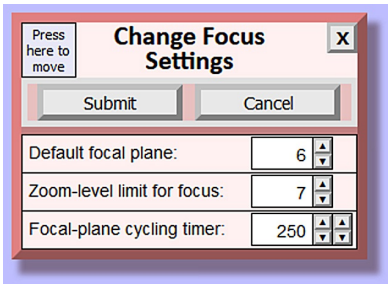
There are two information boxes, and two control "buttons" in the "focus settings" part of the "**Settings**" menu.

1. **"Zoom-level limit" information box.** The "**zoom-level limit**" info-box displays the zoom-level at which focusing becomes enabled ("zoom-level" is equivalent to "magnification": see II.D.3. ("**Changing magnification**", page 5, above). Because focusing up-and-down requires significant computer resources, and because changes in focus usually are not apparent at low magnification, focusing is available only when the zoom-level is greater than or equal to the specified zoom-level. The default is to have focusing available for the four highest zoom-levels, but the user can change this value (see V.A.3.b. (below)).

2. **"Default focal plane" information box.** The "**default focal plane**" info-box shows which focal plane is displayed when viewing the slide at low at low magnification. To conserve computer resources, only one focal plane of a multifocal-plane virtual slide is available for viewing at low magnifications (i.e., when the zoom-level is less than the "zoom-level limit for focusing"; see V.A.1. (above)), and the number of this focal plane is displayed in this information box. Normally, this "default" focal plane is at the center of the stack of focal planes, but the user can change the default focal plane (see V.A.3.a. (below)).

   If the "... & f=value & ..." command is included in the URL to set the focal plane before the slide loads (see II.F.2.d. ("**Command-line arguments: Going to a specific location**", page 15, above)), and if the zoom-level when the slide loads is less than the "zoom-level limit for focusing" (see V.A.1. (above)), then the "default focal plane" is reset to the value indicated in the "f=value " command.

3. **"Change focus settings" button.** Clicking on the "Change focus settings" control causes a box (the "**Change Focus Settings**" box) to open that allows the user to change several variables that are associated with focusing up-and-down through a multifocal-plane virtual slide. The values for these variables can be changed either by clicking on the up/down buttons on the right-side of the text-edit box, or by typing a new value into the text-edit box. After entering the new value, you ***must*** click on the "**Submit**" button for any changes to be effected.



   a. **Change "default focal plane".** Changing (and "submitting") the number displayed in the "default focal plane" text-edit box changes the focal plane that is used at low magnification (see V.A.2. (above)). The value entered must be an integer between zero and the maximum (top) focal plane number (see II.D.4. (page 7, above)). If this setting is changed before a slide is loaded, and if the new value turns-out to be greater than the top focal plane, then (when the slide is loaded) the value for the "default focal plane" is reset to the maximum focal plane.

   b. **Change "zoom-level limit for focus".** Changing (and "submitting") the number displayed in the "zoom-level limit for focus" text-edit box changes the zoom-level at which focusing (for a multifocal-plane virtual slide) becomes available (see V.A.1. (above)). The value entered must be an integer between zero and the number of zoom-levels number (Z_{max}+1; see II.D.3. (page 5, above)). Setting this value to zero causes focusing to be available at all zoom-levels; setting this value to the maximum zoom-level + 1 prevents focusing at all magnifications.

      Note: if you want to turn-off focusing, it is better to use the "**Disable focus**" control (see V.A.4. (below)), instead of using this box with "zoom-level limit for focus" = Z_{max} +1.

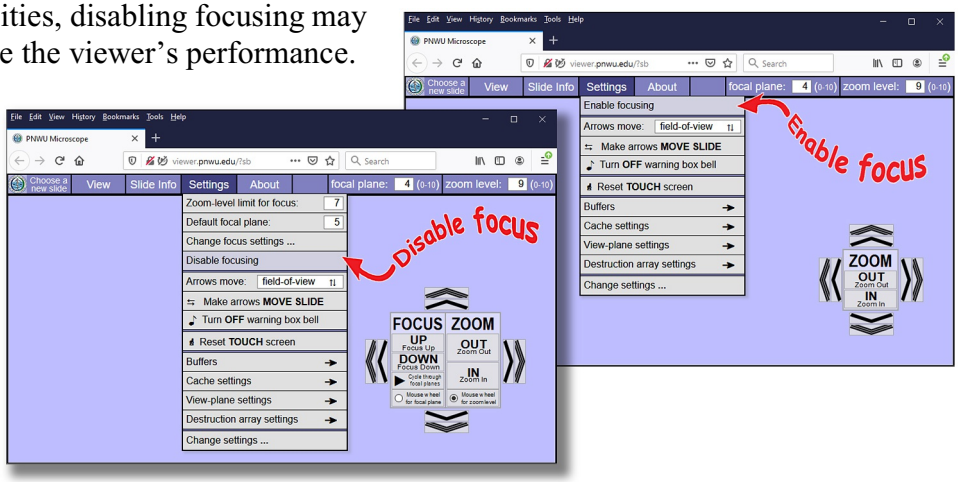
   c. **Change "focal-plane cycling timer".** Changing (and "submitting") the number displayed in the "focal-plane cycling timer" text-edit box changes the rate at which the Viewer repetitively focuses up-and-down through the entire stack of focal planes when the user clicks on the "cycle through focal planes" button on the navigator or the "start focal plane cycling" tab on the "**View**" drop-down menu(see II.D.4.b. ("**Using 'Cycle through focal planes'**", page 8, above)). Normally, the viewer pauses for about ¼ second at each focal plane, but this control allows you to increase or decrease the dwell time that the Viewer spends at each focal plane. The number (which must be an integer greater than 10) entered into this box is the length of time in milliseconds that the viewer pauses at each focal plane.

      If the viewer is actively cycling between focal planes when you change the "focal-plane cycle timer" interval, the change in timing will ***not*** take effect until focal-plane cycling has been stopped and restarted.

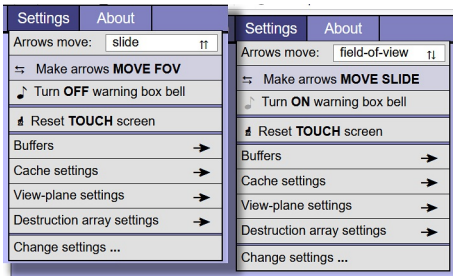
      For the first cycle of repetitive up-and-down focusing, the rate at which the Viewer 'steps' through the focal planes usually is determined by the number and length of "wait" states that the Viewer enters as it downloads the image-tiles for each focal plane (see "**Wait States**" in II.D.4.b. (page 8, above)), rather than by the "focal-plane cycling timer" value. After all of the tiles for the entire 'stack' of focal planes are in memory (i.e., after the first complete (up-and-down) focus cycle), the "focal-plane cycling timer" value becomes rate-limiting and determines the cycling speed.

4. **"Disable focusing" / "Enable focusing" button.** Clicking the "Disable focusing" button on the "**Settings**" drop-down menu causes the Viewer to treat a multifocal-plane slide as if it were a single-focal-plane slide (using the current focal plane as the slide's single focal plane), and changes the button (on the drop-down menu) to an "Enable focusing" button. Clicking the "Enable focusing" button restores focusing capability to the multifocal-plane virtual slide.

When focusing is disabled, the Viewer ignores all of the focal planes except for the current focal plane and the virtual slide behaves as if it were a single-focal-plane slide. Having the ability to focus up-and-down places significant additional demands on the computer's resources (particularly its internet connection). If you are not utilizing the Viewer's focusing capabilities, disabling focusing may improve the viewer's performance.



B. User interactions.



1. **"Arrows move" information box.** This information box ("arrows move:" box) shows whether depressing a move-button (on the navigator or "View" menu) causes the field-of-view or the slide to move in the direction indicated by the arrow (see V.B.2. (below)).

2. **"Make arrows move slide/FOV" button.** Depressing the arrow buttons on the navigator (see II.D.1.a. (page 3, above)) or menu (see II.D.1.b. (page 3, above)) causes the slide to repetitively "step" across the field-of-view (see II.D.2.c. (page 4, above)). Some users want these arrow buttons to move the *field-of-view*, while other users prefer the arrows to move the *slide*. The "make arrows MOVE SLIDE"/"make arrows MOVE FOV" button allows the user to determine which direction the specimen moves when the arrow buttons are depressed.

   In addition to switching the specimen's direction-of-movement, clicking this button toggles the button between being a "make arrows MOVE SLIDE" button and a "make arrows MOVE FOV" button.

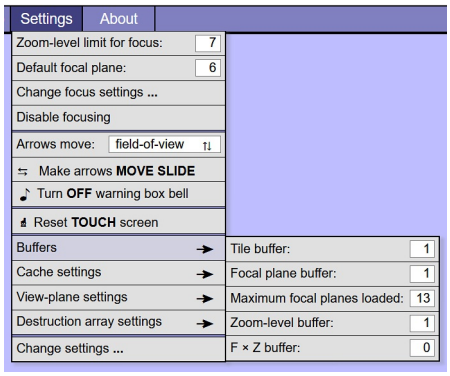
   Moving the slide's *field-of-view* is similar to "scrolling" a page of text; scrolling-*down* a page of text causes more lines of the text to appear at the bottom of the page. When scrolling-down a page, you are moving your field-of-view downwards. Scrolling the field-of-view is equivalent to keeping the field-of-view constant and moving the specimen (or a page of text) in the opposite direction within the field-of-view. Thus, if the navigator's/menu's arrow buttons move the *field-of-view*, activating an UP button decreases the y-coordinate of the center of the screen; the field-of-view is "moving" *up* or, equivalently, or (equivalently) the specimen is "moving" *down*. Conversely, activating a DOWN button increases the y-coordinate of the center of the screen (which "moves" the field-of-view *down*, or the specimen *up*). Similarly, activating a LEFT button decreases the x-coordinate of center of the screen (i.e., it moves the specimen to the *right*), and activating a RIGHT button increases the x-coordinate of the center of the screen.

3. **"Turn ON/OFF warning bell" button.** The Viewer was designed to have an aural warning ("beep") accompany display of a warning box (see VIII. (page 36, below)). During beta-testing of the Viewer, most users found this "beep" annoying, so the default is to ***not*** have a "beep" when a warning is issued. However, it is possible to turn this feature on. The "turn OFF warning bell"/"turn ON warning bell" button allows the user to determine whether there is a audible warning "beep" when a "warning box" is displayed; clicking this button toggles the "beep" off/on.

4. **"Reset TOUCH screen" button.** For a touch-screen device, the Viewer needs to account for all of the fingers that are touching the screen, and if the Viewer loses track of a finger, it most likely will start sending the user error messages. If the Viewer starts sending you a bunch of "touch-point errors", clicking this "Reset TOUCH screen" button might get the Viewer back on track (clicking the "Reset TOUCH screen" button clears the arrays in which the viewer has stored the touch-point information, and forces the viewer to build a new inventory of the touch-points).

   If you need to use the "Reset TOUCH screen" button, please report this error (see I.B. ("**Talking to us**", page 1, below)). If clicking the "Reset TOUCH screen" button does *not* stem the stream of "touch-point" error-messages, you probably will need to exit and re-invoke the viewer (see II.A. ("**Invoking the viewer**", page 1, above)).

C. **Technical settings.** This section describes controls and information boxes used to debug and correct issues that affect the Viewer's performance. It is unlikely that you will need any of the items described in this section (unless you are writing computer code for this open-source project), and you should discuss your viewer's problems with us (see I.B. ("**Talking to us**", page 1, below)) before recourse to the tools described in this section.

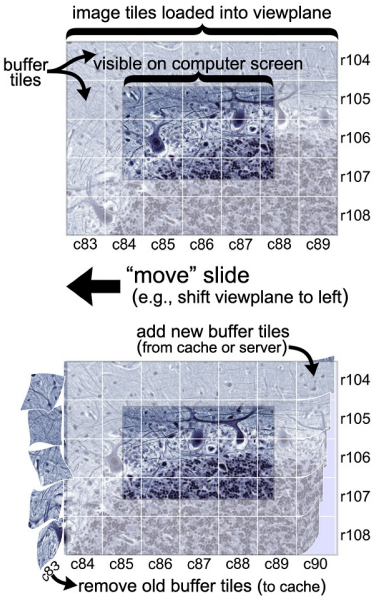


1. **"Buffers ➜".** Moving the mouse cursor over the tab labeled "**Buffers ➜**" (or touching the tab with your finger on a touch-screen device) causes a side-menu to appear that lists the values of variables that are involved in creating buffers that improve the Viewer's responsiveness to user actions.

   In order to decrease the time that the user must wait while image-tiles are loaded, the Viewer attempts to anticipate the user's needs by preloading a rim of "buffer tiles" around the region that currently is being displayed, and by preloading view-planes for zoom-levels and focal planes adjacent to the current view-plane.[11] The number of image-tiles and the number of view-planes included in these buffers are shown in this side menu.

[11] A "view-plane" is a software object that the Viewer uses to display a region of a specific zoom-level and focal plane of a virtual slide. The view-plane contains a grid of image tiles (including the buffer tiles); all of these image tiles are from the same zoom-level and focal plane. When the user "moves" the specimen across the screen, the user actually is changing the location of the view-plane's grid relative to the top, left corner of the computer screen. In order to be responsive to the user, the Viewer has a stack of view-planes, each at a different zoom-level or focal plane, and all aligned in their x,y-axes. At any given time, only one of the view-planes in this stack is visible to the user; the other view-planes are hidden, waiting to be displayed if the user changes the zoom-level or focal plane.

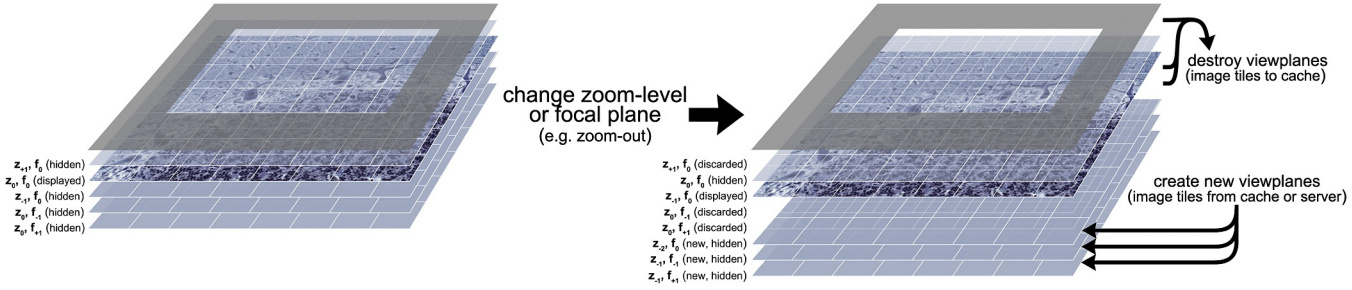
The information listed in this side menu is intended primarily for diagnostic purposes if the user needs to contact us (see I.B. ("**Talking to us**", page 1, below)) regarding problems with the Viewer's interaction with the server. The values of these buffer variables can have a significant impact on the Viewer's responsiveness to user actions. If the buffer size is too small, the user will have to wait while image tiles (for the current view-plane) are obtained from the server. If the buffer size is too large, the user will have to wait while the viewer handles the increased size and number of (hidden) view-planes. The values for these variables can be changed (see X. ("**Changing settings**", page 41, below)), but this should be done *only* after talking with (and with the approval of) the network administrator.



a. **"Tile buffer".** The value in this information box is the number of image tiles (on each edge of each view-plane) that form a rim surrounding the field-of-view. As the specimen is moved, new image-tiles are added to one side of the view-plane and removed from the opposite side, keeping the number of buffer tiles in this hidden 'rim' of tiles approximately constant (at the value indicated in the information box in the side menu) In most cases, the optimum value for this variable seems to be 1.

b. **"Focal plane buffer".** The variable whose value is shown in this information box determines the number of focal planes that are included in the view-planes that are pre-loaded into memory during non-focus operations. In most cases, the optimum value for this variable is about 1.

In addition to a buffer of image tiles (at the current-zoom level and focal plane) that attempt to anticipate the user moving the specimen (see V.C.1.a. ("**Tile buffer**", above)), the Viewer also attempts to anticipate the user changing the focus (for a multifocal-plane slide) by pre-loading views of other focal planes (at the same zoom-level and aligned in the x,y-axes with the current view). When the user focuses "up", the Viewer hides the old view-plane and displays the pre-loaded view-plane whose focal plane is one more than the old focal plane. Conversely, when the user focuses down, the current view-plane is hidden and the pre-loaded view-plane whose focal plane is one less than the old focal plane is made visible.



The "focal plane buffer" value is the number of view-plane within the 'stack' of available view-planes that are at the same zoom-level as the current view-plane and whose focus is on one "side" of the focal plane of the current view-plane (i.e., either the focus above the current focal plane, or alternatively, is below the current focal plane). If n is the value for "focal plane buffer" (i.e., the value shown in this window), then there will be a maximum of $(2 \times n) + 1$ focal planes represented in the 'stack' of view-planes (the number of focal-planes in the "stack" will be less than this maximum when the current (visible) focal plane is near the top or bottom of the focus range).

c. **"Maximum focal planes loaded".** As this information box's name indicates, the value shown in this box is the maximum number of focal planes that will be included in the 'stack' of available view-planes during a focus-operation (for the definition of "view-plane", see footnote 11 (above)).

For non-focus operations, such as moving the specimen across the screen, it is necessary to limit the number of focal-planes in the 'stack' of available view-planes (see V.C.1.b. ("**Focal plane buffer**", above)) because the Viewer can become balky and non-responsive if it tries to "move" (and update) too many view-planes at one time. However, there are many times when the user will want to focus up-and-down through the specimen without changing the specimen's position on the screen. For this type of focusing operation, the Viewer will be most responsive if it can create a large 'stack' of view-planes at different focal planes (the large 'stack' in this case is not a problem since this operation does not involve "moving" the 'stack' of view-planes, so the Viewer is not adding/removing image tiles from existing view-planes). Thus, when solely focusing up-and-down, the Viewer ignores the "focal plane buffer" limit and adds view-planes up the the limit specified by "maximum focal planes loaded". If the user had been focusing up-and-down, when the user next initiates a non-focusing operation (such as "moving" the specimen across the screen), the Viewer immediately "restricts" the number of focal-planes in the 'stack' (see V.C.3.d (page 31, below)) to the number allowed by the "focal plane buffer" variable.

The value for "maximum focal planes loaded" cannot exceed the total number of focal planes in the specimen (when a slide is loaded), although the value for "maximum focal planes loaded" can be less than the number of focal planes in the specimen. Normally, (i.e., the user does ***not*** use the "**Change settings ...**" option (see V.C.5. (page 33, below)) to specify a value for "maximum focal planes loaded"), the value for "maximum focal planes loaded" is set automatically to the total number of focal planes in the specimen. However, if the number of focal-planes is very large (e.g., slide #UM237-3), there can be a problem with over-filling the destruction array (see V.C.4. ("**Destruction arrays**", page 31, below)). In this case, the "maximum focal planes loaded" automatically defaults to the "maximum destruction array size" (unless it has been changed by the user; see V.C.5. ("**Change settings...**", page 33, below)).

If the user sets "maximum focal planes loaded" to a value that is greater than the "maximum destruction array size" (see V.C.4. (page 31, below) for more about the destruction array), and the specimen is focused up-and-down (so that the number of focal planes in the 'stack' of view-planes equals the "maximum focal planes loaded" value), the destruction array probably will exceed its allowed size when the user initiates an action other than focusing (and the Viewer decreases the number of focal planes in the 'stack' of view-planes to that specified by the "focal plane buffer" value; see V.C.1.b. (above) & V.C.3.d. (page 31, below)). In this case, an "alert box" message is displayed that reports that the destruction array is too large, and the system pauses (usually for a very short (seconds or milliseconds) interval) while the view-planes in the destruction array are destroyed.

It is possible (although not recommended) for the user to change the "maximum focal planes loaded" to a user-specified value before a slide has been loaded into the Viewer (see V.C.5. (page 33, below)). If, after the slide has been loaded, it turns out that the user-specified value for "maximum focal planes loaded" is greater than the number of focal planes in the virtual slide, the value for "maximum focal planes loaded" will be reset to the specimen's number of focal planes (since "maximum focal planes loaded" must be less than or equal to the number of focal planes in the specimen), and a warning-box indicating this change is displayed.

It is unlikely that you will encounter a situation where you would benefit by changing the value of the "maximum focal planes loaded" variable. There are very few situations where a user-specified value for this variable would be helpful, particularly since the image-tiles from destroyed view-planes (view-planes would be destroyed if the maximum number of focal planes is exceeded) are moved to the cache, from which they can be retrieved and re-used (see V.C.2 (page 25, below)). Conceivably, if the "cache size" has been set (by the user)

to a small value (see V.C.2.a. (page 25, below)), or if the screen displaying the specimen is extremely large, *and* if the specimen has very many focal planes (so that "maximum focal planes loaded" is limited by a "maximum destruction array size" value that is much smaller than the number of focal planes), then it is possible that image-tiles which the Viewer needs (to rebuild a focal plane that had been destroyed) might be discarded from the cache (to keep cache size less than its maximum) before the Viewer tries to retrieve them; in this (unlikely) scenario, increasing the value for "maximum focal planes loaded" might improve Viewer performance when quickly focusing up-and-down. Similarly, if a specimen, in which "maximum focal planes loaded" value is less than the number of focal planes, focuses through the focal planes *very* quickly (by setting the value for"focal-plane cycling timer" to a *very* small value; see V.A.3.c. (page 19, above)), it *might* be useful to set maximum focal planes loaded = *number of focal planes*, because that would obviate the need for the Viewer to have to destroy and rebuild the view-planes during the up-and-down focusing cycle (and it would avoid the possibility that the previous instance of a discarded view-plane was still in the destruction array (waiting to be destroyed) when the Viewer needed to re-create that view-plane). However, unless the computer is incredibly slow or the rate of cycling is incredibly fast, it is unlikely that rebuilding view-planes from cached image-tiles would be rate limiting, so even in this scenario, it is unlikely that changing "maximum focal planes loaded" would improve Viewer performance noticeably.

d. **"Zoom-level buffer".** The variable whose value is displayed in this window is the zoom-level's equivalent of the focal plane's "focal plane buffer" value described above (see V.C.1.b. (above)). The "zoom-level buffer" value is the maximum number of view-planes (for "view-planes", see footnote 11 (page 21, above)) in the 'stack' (of view-planes) whose zoom-level either is greater than or, alternatively, is less than the zoom-level of the currently visible view-plane (and whose focus is at the same focal plane as the current focal plane).

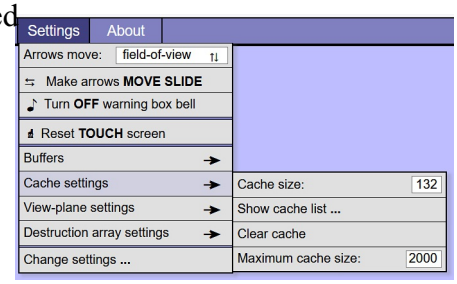
By having a buffer of pre-loaded zoom-levels, the Viewer is more responsive when the user changes magnification. Because of this buffer, when the zoom-level changes, the Viewer can immediately display the (previously hidden) pre-loaded zoom-level, rather than making the user wait while image-tiles are obtained from the server. While the user examines the newly displayed view-plane, the Viewer is working in the background to build another view-plane (at the next zoom-level), so it will have a new zoom-level view-plane pre-loaded and ready if the user changes the zoom-level again. The number of zoom-levels available is kept (more-or-less) constant at: 2 × (zoom-level buffer-size) + 1; as a result, in addition to creating a new view-plane, the Viewer (working in the background) also destroys an old view-plane (see V.C.4. (page 31, below)).

Because obtaining image-tiles from the server can be very slow, it is possible for the user to change zoom-levels (or focal planes) faster than the Viewer can create new view-planes. If this happens, the Viewer enters a "wait" state (see discussion (page 8, above) in II.D.4.b. ("**Cycle through focal planes**"), and V.C.3.c ("**Missing tiles**", page 31, below)), in which the Viewer is not responsive to the user's commands, when it tries to display a view-plane which is missing image-tiles. This "wait-state" persists until the pending view-plane has completely loaded.

There is an optimum size for the zoom-level and focal plane buffers. Having a large zoom-level buffer or focal plane buffer can be just as detrimental to the Viewer's performance as having too small of a buffer, since all of the view-planes in the Viewer's 'stack' have to be updated whenever the user moves the specimen. If the buffer is large (i.e., there is a large 'stack' of available view-planes), the Viewer can become balky and movements can become jerky because the Viewer may still be updating (hidden) view-planes in the 'stack' when the user tries to initiate the next action. Normally (depeding on internet and computer speeds), if the user spends more than ~½ second looking at a view-plane before changing focus or magnification, then the optimum value for "zoom-level buffer"(and for "focal plane buffer") most likely will be one.

e. **"F × Z buffer".** *The value shown in this box should be* ***zero****.* **Do NOT change this value.** The variable whose value is shown in this box controls the number of off-axis view-planes in the Viewer's 'stack' of available view-planes (i.e., view-planes where both the focal plane and the zoom-level are different from the current (visible) view-plane). This variable is intended principally for testing and debugging new versions of the Viewer. Increasing the value of this variable will greatly increase the demands on the Viewer (and network bandwidth) with little or no benefit.

2. **"Cache ➜".** Moving the mouse cursor over the tab labeled "Cache settings ➜" (or touching the tab with your finger on a touch-screen device) causes a side-menu to appear that contains information and tools related to the Viewer's image cache. The number of items in this side-menu depends on whether there are image-tiles in the cache. The "show cache list" and "clear cache" buttons are displayed only if there are image-tiles in the cache.



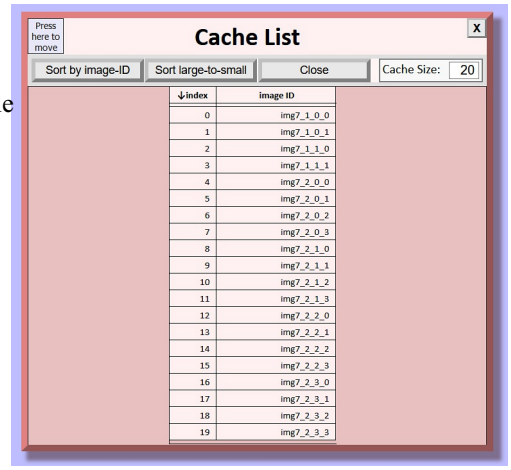
The image of the slide that is displayed by the Viewer actually is an array of smaller, square 'pieces' of the image, known as "image-tiles", that are inserted into a 'frame', known as a "view-plane". As described earlier (see V.C.1.a. ("**Tile buffer**", page 22, above)), when the user "moves" the specimen across the screen, image-tiles are added to one side of the view-plane and removed from the other side. Since it can take considerable time for the Viewer to get image-tiles from the server and since the user frequently will return to a previous view of the specimen, the Viewer saves the image-tiles that it removed from the view-plane in a "cache" instead of deleting these image-tiles from memory.

Similarly, as described earlier (see V.C.1.b ("**Focal plane buffer**", page 22, above) & V.C.1.d ("**Zoom-level buffer**", above)), the view-plane that is visible to the user is part of a 'stack' of available view-planes, and new view-planes are created and old view-planes are destroyed when the user changes focus or magnification. Each of these view-planes contains an array of image-tiles; when a view-plane is destroyed, the image-tiles from that view-plane are saved in the image cache, so that they can be re-used if the user later returns to this part of the specimen.

Before requesting an image-tile from the server, the Viewer 'looks' in the cache to see if there is a copy of the image-tile is in the cache. If the image-tile that the Viewer needs is in the cache, the Viewer extracts the image-tile from the cache and uses it (instead of getting a new copy of the image-tile from the server).

a. **"Cache size".** The value in this box is the number of image-tiles "currently" in the cache. The value displayed in this information box does ***not*** automatically update when the cache size changes, so the value shown here is the size of the cache when the user moved the mouse cursor over (or, for a touchscreen device, touched with their finger) the "**Settings**" tab on the main menu. However, since the mouse (or the user's finger) has to be on the specimen (or on the navigator or on "**View**" menu) for all changes to the image of the specimen except focus-cycling, the cache size should not change during the time while the user is viewing this information box unless focus-cycling is on when the user moves the mouse to the "**Settings**" drop-down menu. If the user moves the mouse off of the "**Settings**" drop-down menu (or, for a touch-screen device, touches some other part of the Viewer's window), the "**Settings**" drop-down menu closes, and the value in the "cache size" box will be updated if the user re-opens the menu (by moving the mouse over the "**Settings**" tab on the main menu).

b. **"Show cache list …".** This button is only visible if there are image tiles in the cache. Clicking on this button opens a movable box ("**Cache List**"; movable boxes are described in VII. (page 34, below)) that contains a table with the id's of all of the image-tiles in the cache.[12] This list is intended primarily as a diagnostic tool when modifying the javascript code that controls the Viewer; it is unlikely that most users would ever need to look at these data. To close the box, click on the "Close" button on the box's menu (or click on the "**X**" button in the box's upper-right corner ("click" is defined in footnote 2 (page 5, above))).



The "**cache list**" box will remain open until the user closes it (by clicking on the "Close" button on the menu or on the "**X**" button in the box's upper-right corner), but the list in the table does ***not*** update. As a result, the list does ***not*** correspond to the current status of the cache, but rather to the status of the cache when the box was opened. In the table, "index" is the order in which the image-tiles were added to the cache (with "0" being the oldest image-tile in the list (i.e., the first of the image-tiles currently in the cache to have been added to the cache)).

Clicking on the buttons on the left side of the box's menu causes the list to be sorted either by "index" value (so you can see the order in which the image-tiles they were added to the cache) or by image-tile "id" value or (so you can quickly see if particular tiles are in the cache), and determines whether the sorted list is displayed in ascending or descending order. The arrow in the list's heading indicates the variable on which the list is sorted and the direction in which it list was sorted. Remember that these "sort by" buttons are sorting (or re-sorting) a static list which represents a snap-shot of the cache at the time the "cache list" box was opened; *the cache list is **not** update when the list is sorted*. The "Press here to move" button can be used to drag this box ("**Cache list**" box) to a different location in the Viewer's window (see VII. (page 34, below)).

c. **"Clear cache".** Clicking on this button removes all of the image-tiles in the image cache from the Viewer's memory. In addition to the Viewer's image-tile cache, your internet browser may also have a cache, which is independent of the Viewer's cache. If you need to force the Viewer to obtain a new image-tile from the server (a computer programmer re-writing the Viewer's code probably is the only person who would want to do this), you probably will need to clear both the Viewer's cache ***and*** the browser's cache.

After clearing-out the cache, the cache size = 0 (i.e.,there are no image-tiles in the cache). As a result, the Viewer hides the "**Show cache list**" and "**Clear cache**" buttons after the "**Clear cache**" button has been clicked (until more image tiles are dumped into the cache).

d. **"Maximum cache size".** As the title implies, the value in this box is the maximum size (number of image-tiles) to which the Viewer's image-tile cache is allowed to grow. There are limits to a computer's resources, so the image cache cannot be allowed to become infinitely large. After the cache reaches the size indicated in this box, when the Viewer adds a new image-tile to the cache, the Viewer keeps the cache size constant by removing from the cache (and from memory) the image-tile that has been in the cache the longest (i.e., the image tile whose "index" is 0 in the "cache list"; see V.C.2.b. (above)).

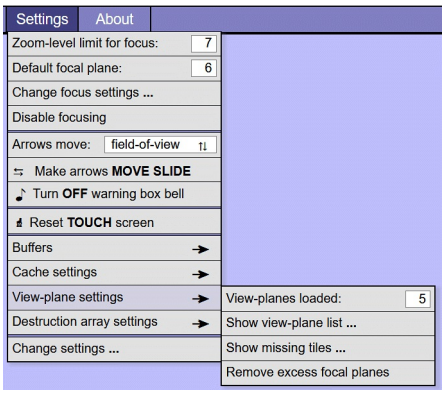
[12] Each object in HTML can be assigned a text-string known as the object's "id". The image tiles used by Viewer are <img> tags. This "id" uniquely identifies the image-tile and its location within the specimen (<img id="img*f*_*z*_*x*_*y*">, where *f*, *z*, *y*, and *x* identify the tile's focal plane, zoom-directory-level, y-subdirectory-number and x-tile(jpg)-number, respectively).

Its effect on the Viewer's performance makes using image cache worthwhile. However, this cache can require a more-than-trivial amount of computer memory. Each image-tile requires about 64kB of memory, so using the default value for maximum cache size (2000 image-tiles; the maximum size of the image cache can be changed; see V.C.5. ("**Change settings**", page 33, below)), the cache will use approximately 125MB of memory. Most modern personal computers have sufficient memory, and memory consumption by the Viewer's image cache (using the default value for maximum cache size) has not seemed to be a problem. On the other hand, each instance of the Viewer has its own cache, so it probably is a good idea to limit the number of copies of the Viewer that are open at one time (in other words, if you've got the Viewer open in multiple tabs in your browser (or in multiple browser windows), you should close the copies of the Viewer that you are no longer using).

Conceivably, an extremely large cache size might have a negative impact on Viewer performance (even if the amount of computer memory is unlimited) because, when the Viewer needs a new image-tile, the Viewer reads the "id" of every tile in the cache before it decides to send a request to the server (for the jpeg used in the image-tile). In most cases, the software that the browser uses to read the id's from an array of HTML tags should be sufficiently efficient so that the computer's memory limitations, rather than the time required to read through the cache, most likely would be what determines the optimal size for the cache. However, extracting the "id" from all the image-tiles in the cache, and comparing this string with the needed image-tile's "id", could become rate limiting if the cache was extremely large and/or the computer very slow (or the browser's software very inefficient).

The optimal size for the cache will depend on the individual circumstances: e.g., computer memory available, memory requirements other programs that are running concurrently (including other instances of PNWU Virtual Microscope Viewer), how the slide is being studied (the cache is only helpful if the user returns to the same location, magnification, and focus level that have been visited recently enough so the image-tile is in the cache), the bandwidth of the computer's internet connection to the server, and the server's response time. A computer with a large amount of memory and a slow internet connection may benefit from increasing the cache size (particularly if the user was interested in a limited region of the slide), while a computer with limited memory and a fast internet connection might benefit from a reduced cache size. Currently the "**Change settings**" box (see V.C.5. (page 33, below)) prevents the user from setting the cache size to greater than 40,000 image-tiles, and warns the user if the user attempts set the cache size to greater than 4,000 image-tiles; however these values were chosen more-or-less arbitrarily.

3. **"View-plane settings ➜".** Moving your mouse over this tab in the "**Settings**" drop-down menu (or touching this tab with your finger on a touchscreen device) causes the Viewer to display a drop-down side-menu allows you to access information about the Viewer's view-planes.



View-planes were defined earlier in this document (see footnote 11 (page 21, above)), and a 'stack' of view-planes (technically, an array of view-plane objects) forms the heart of the Viewer. However, unless you are a computer programmer writing code for the Viewer, you don't need to know anything about view-planes, and the only time you might need to look at the information accessible through this side-menu would be if you encountered a computer bug and (after you told us about the bug; see I.B. ("**Talking to us**", page 1, above)) we asked you to send to us some information from one of the boxes available through this menu.

a. **"View-planes loaded".** This box shows the number of view-planes that currently are in the 'stack' (this value is *not* a static snap-shot; it is updated whenever the number of view-planes changes). The 'stack' of view-planes is a buffer that is used to improve the Viewer's responsiveness by anticipating changes in zoom-level or focus (see V.C.1 (page 21, above)). The number of view-planes in the 'stack' (i.e., "view-planes loaded") normally is determined by the values shown in the "zoom-level buffer" box (see V.C.1.d (page 24, above)), and the "focal plane buffer" box (see V.C.1.b (page 22, above)) or "maximum focal planes loaded" box (see V.C.1.c (page 23, above)).

Usually, the value in the "view-planes loaded" box will be:

➢ view-planes loaded = (2 × zoom-level buffer) + 1, *if* zoom-level < zoom-level limit for focusing (see V.A.3.b. (page 19, above)), and zoom-level is not near its limits (i.e., zoom-level ≥ zoom-level buffer, *and* zoom-level < (number of zoom-levels − zoom-level buffer)).

➢ view-planes loaded = (2 × zoom-level buffer) + (2 × focal plane buffer) + 1, *if* the last operation was *not* changing the focal plane, (and zoom-level ≥ zoom-level limit for focusing), and zoom-level is not near its limits (i.e., zoom-level ≥ zoom-level buffer, and zoom-level < (number of zoom-levels − zoom-level buffer)), and the focus is not near its limits (i.e., focal plane ≥ focal plane buffer, and focal plane ≤ (highest focal plane − focal plane buffer)).

➢ (2 × zoom-level buffer) + (2 × focal plane buffer) + 1 ≤ view-planes loaded ≤ maximum focal planes loaded, *if* the last operation was changing the focal plane. The first argument in this inequality: (2 × zoom-level buffer) + (2 × focal plane buffer) + 1, is not accurate (it's too large) if zoom-level is near its limits (i.e., zoom-level ≥ zoom-level buffer, *or* zoom-level < (number of zoom-levels − zoom-level buffer)), or the focus is near its limits (i.e., focal plane ≥ focal plane buffer, *or* focal plane ≤ (highest focal plane − focal plane buffer)). If the last set of operations was to focus the specimen through *all* of it's focal planes (e.g., see II.D.4.b. ("**Using "Cycle through focal planes"**", page 8, above)), then the value for "view-planes loaded" will be: view-planes loaded = maximum focal planes loaded.

b. **"Show view-plane list ...".** Clicking on this button opens a movable box (the "**View-plane list**" box; movable boxes are described in VII. (page 34, below)) that contains a table showing data for each view-plane that was in the 'stack' of view-planes when the user clicked on this button. This table ***does not update***; the data in this table is a snap-shot of status of the 'stack' of view-planes at the time the user clicked on this button.

Press here to move

View-plane List

X

Sort by F then Z | Sort large-to-small | Close | Number of view-planes loaded: 5

is visible	↓index	F (focal plane	Z (zoom level	zoom multiplier	in viewing frame: start tile, x-value	in viewing frame: number of tiles, x-axis	in viewing frame: start tile, y-value	in viewing frame: number of tiles, y-axis	in viewing frame: left offset (pixels)	in viewing frame: top offset (pixels)	in database: start tile, x-value	in database: number of tiles, x-axis	in database: start tile, y-value	in database: number of tiles, y-axis
☐	0	6	7	8	85	8	80	5	-431	-233	1	126	29	70
☑	1	6	8	4	175	8	162	5	-348	-282	2	252	58	140
☐	2	5	8	4	175	8	162	5	-348	-282	2	252	58	140
☐	3	7	8	4	175	8	162	5	-348	-282	2	252	58	140
☐	4	6	9	2	354	8	325	5	-438	-637	4	504	116	280

The "**View-plane list**" movable box has its own menu. In addition to a "**Close**" button (which closes the box), this menu contains two buttons that allow the user to sort the rows of this table. The leftmost button (the "**Sort by**" button) determines whether the entries in the table are sorted by the view-planes' index (see V.C.3.b.(2). (below)), focal plane (see V.C.3.b.(3) (below)), or zoom-level (see V.C.3.b.(4) (below)). Clicking on this button cycles through the three options ("Sort by index", "Sort by F then Z", and "Sort by Z then F"). Within the table's heading, an arrow marks the variable on which the table is sorted. The second button on the "**View-plane list**" box's menu toggles between "Sort large-to-small" and "Sort small-to-large", and determines the order in which the entries in the table are sorted. The direction of the arrow in the table's heading indicates the direction in which the table currently is sorted.

Each row of the table is data from a view-plane. The elements in the table displayed in this box are:

(1) **"is visible".** The check-box indicates which of the view-planes was visible at the time the table was created.

Only one view-plane can be visible (*view-plane-node*.style.visibility = "visible") at any given time; all the other view-planes must be hidden (*view-plane-node*.style.visibility = "hidden"). The "visibility" property, rather than the "display" property (*node*.style.display = "grid"/"none"), was used because of an issue with the grid displaying correctly when the "display" property was toggled (in retrospect, 1-year *ex-post facto*, I'm not sure that I didn't try to set *node*.style.display = "block", which might be the reason that the view-plane did not display correctly).

(2) **"index".** This is the view-plane's index within the 'stack' (array) of view-planes. Since new view-planes are added to the 'top' of the array, this also is the order in which the view-planes were created, with index = 0 belonging to the 'oldest' view-plane. When a view-plane is removed from the 'stack' it is spliced-out (*array*.splice(*index*,1);), so there are no 'gaps' in the numbers assigned to "index".

(3) **"F".** As the sub-heading indicates, this is the focal plane of the view-plane (see II.E.7.c. (page 13, above)). Although only one view-plane in the 'stack' can have a unique combination of "F" and "Z" (see footnote 11 (page 21, above)), there can be multiple view-planes with the same value of "F" (but with different values of "Z").

(4) **"Z".** As the sub-heading indicates, this is the zoom-level of the view-plane (see II.E.7.b. (page 12, above)). Although only one view-plane in the 'stack' can have a unique combination of "Z" and "F" (see footnote 11 (page 21, above)), there can be multiple view-planes with the same value of "Z" (but with different values of "F").

(5) **"zoom multiplier".** This value is $2^{(Zmax - Z)}$, where "Zmax" is the maximum zoom-level and "Z" is the zoom-level of the view-plane. When the user "moves" the slide, the Viewer adjusts the position of each view-plane in the 'stack' (regardless of whether it is visible) relative to the upper-left corner of the Viewer's slide window. The amount that each view-plane is moved is calculated in screen-pixels (since the pixels on the image-tiles and the coordinates of the upper-left corner of the Viewer's window (or any other location on the screen) are in screen-pixels; for more about screen-pixels *vs.* specimen-pixels, see footnote 5 (page 12, above)). However, moving 1 pixel on a view-plane with zoom-level = $\mathbb{Z}$ is equivalent to moving 2 pixels on a view-plane with zoom-level = $\mathbb{Z}$+1, or moving 4 pixels when zoom-level = $\mathbb{Z}$ + 2. The Viewer uses the ratio of the "zoom multiplier" values when calculating how much to "move" each view-plane.

(6) **Database grid parameters.** The columns on the right-side of the table contain information about the image-tiles for the entire specimen (i.e., image tiles in the server's database). There are four parameters: "start tile, x-value", "number of tiles, x-axis", "start-tile, y-axis", and "number of tiles, y-axis".

At each zoom-level, the image-tiles are identified by integers indicating the location of the image-tile in a grid that covers the entire specimen (the tiles are numbered, along the x- and y-axes, relative to the "absolute" upper-left corner of the specimen at the view-plane's zoom-level). The "absolute" upper-left corner is the upper-left corner of the zoom-level = 0 tile. Since the scanned specimen never (or almost never) shrinks (in 2^Z- size steps) to fit exactly into the single 256×256-pixel tile that forms the Z=0 view-plane, as the zoom-level increases, there will be "empty" tiles that fit into the space between the "absolute" upper-left corner and the actual upper-left corner of the specimen. These "empty" image-tiles are not stored in the database, but they are included when "counting" tiles to determine the x,y-integers that identify each image tile. Thus, the first image-tile at a zoom-level may have an x-value (integer indicating tile location on the horizontal axis) greater than 0 if there are "empty" tiles located to the left of the specimen (between the actual specimen and the "absolute" left side). Similarly, this first tile may have a y-value (integer indicating tile location on the vertical axis) greater than 0 if there are "empty" tiles located above the specimen (between the top of the specimen and the "absolute" top). The "**in database**" parameters: "**start tile, x-value**" and "**start-tile, y-value**" are these x,y location-values (integers for numbering tiles) for the tile that is the top-left tile of the entire specimen at the view-plane's zoom-level.

After the specimen has been scanned, an image of the specimen at each zoom-level is constructed and then is chopped into square image-tiles (each tile is 256×256 pixels). The number of rows and columns of image-tiles is dependent on the dimensions of the scanned specimen and the zoom-level. The "**in database**" parameters: "**number of tiles, x-axis**" and "**number of tiles, y-axis**" are the number of columns (x-axis) and rows (y-axis) of image-tiles for the entire actual specimen at the view-plane's zoom-level (not including "empty" tiles above or to the left of the actual specimen).

(7) **View-plane grid parameters.** These parameters are found in the columns in the middle of the "**View-plane List**" table. There are six view-plane grid parameters: "start tile, x-value", "number of tiles, x-axis", "start-tile, y-axis", "number of tiles, y-axis", "left offset" and "top offset".

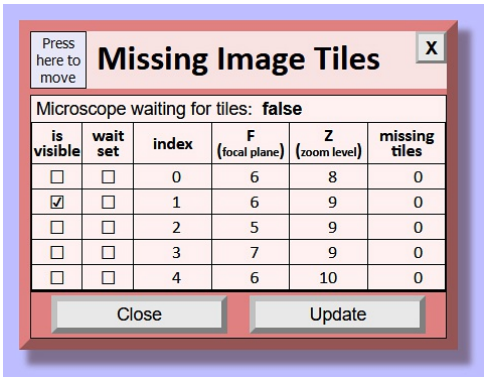
When the Viewer is first loaded, and whenever the Viewer's window is resized, the Viewer calculates the number of image tiles (256×256 pixels) that would completely fill the Viewer's specimen window. When a view-plane is created, the Viewer compares the number of image-tiles needed to fill the Viewer's window (plus the"buffer tiles"; see V.C.1.a. (page 22, above)) with the number of image-tiles in the entire specimen (at the view-planes zoom-level; the number of tiles in the specimen are the "in database" values for "number of tiles, x-axis" and "number of tiles, y-axis"; see V.C.3.b.(6). (above)). If the specimen is larger than the horizontal aspect or vertical aspect of the Viewer's window (including the buffer), then the view-plane is constructed with enough columns and/or rows to completely fill the Viewer's window plus twice the "buffer tile" value (so there is a buffer on the left & right sides, and top & bottom sides; see V.C.1.a, (page 22, above)). If the entire specimen (in the horizontal and/or vertical axis at the view-plane's zoom-level) is smaller than the Viewer's window (plus the buffer tiles), then the view-plane is constructed with enough columns and/or rows to fit the entire specimen.

The value in the table in the "**View-plane List**" movable box for "**in viewing frame: number of tiles, x-axis**" is the number of columns of image-tiles in the view-plane, and the value for "**in viewing frame: number of tiles, y-axis**" is the number of rows of image tiles in the view-plane. At high magnification, the specimen is larger than the Viewer's window, so all high-magnification view-planes (i.e., high zoom-level values) will have the same value for "in viewing frame: number of tiles, x-axis" and the same value for "in viewing frame: number of tiles, y-axis" (this is the number of tiles that fit into the Viewer's window + buffer). For low-magnification view-planes (where the entire image fits into the Viewer's window), the values for "in viewing frame: number of tiles, x-axis" and "in viewing frame: number of tiles, y-axis" will be equal to "in database: number of tiles, x-axis" and "in database: number of tiles, y-axis", respectively.

The values in the "**View-plane List**" movable box for "**in viewing frame: left offset**" and "**in viewing frame: top offset**" are the coordinates (in screen-pixels) of the top-left corner of the view-plane relative to the top-left corner of the Viewer's window. When the user "moves" the slide, the 'stack' of view-planes is shifted relative Viewer's window; this shift in the slide's position is achieved by changing the "top" & "left" values of the view-plane's grid of image tiles (technically, this grid is an HTML tag that is part of the view-plane object). The information in the "**View-plane List**" table is a static snap-shot of the status of view-plane 'stack' at the time the "**View-plane List**" box was opened, so in order to see what happens to the view-planes when the slide is moved, you would need to note the values in the table, close the box (by clicking on the "Close" button on the menu or on the "**X**" button in the box's upper-right corner), and then, after "moving" the specimen, re-open the box (by clicking on the "**Settings**" menu → "**View-plane settings**" → "**Show view-plane list ...**").

The values for "left offset" and for "top offset" should be the same for all view-planes that are at the same zoom-level (if they don't have the same "left/top-offset" values, clicking the "View" menu → "Realign focal planes" button (see III.D. (page 17, above)) should force the all view-planes at the current zoom-level to have the same "left offset" and "top offset" values (remember that you have to close and re-open the "Show view-plane list" box to update the table)). The "left offset" and for "top offset" values for view-planes at different zoom-levels will be different, but except for rounding errors, these should differ by multiples of 256 after accounting for the magnification-multiplier (see V.C.3.b.(5). (page 29, above)) and for changes in "start tile" values (see below).

The values in the "**View-plane List**" movable box for "**in viewing frame: start tile, x-value**" and "**in viewing frame: start tile, y-value**" are the x,y-location values for the image-tile that is in the upper-left corner of the view-plane (for an explanation of the image-tile location values, see V.C.3.b.(6). (above)). As the specimen is moved across the computer screen, image-tiles are added to, and removed from, the view-plane in order to maintain a buffer of image tiles around the edges of part of the specimen that is displayed on the computer screen (see V.C.1.a. (page 22, above)). Thus, as the slide is moved across the computer screen, the tile in the upper-left corner of the view-plane will change whenever the "tile-buffer offset" (see X.B.2. (page 43, below)) is exceeded and a different image-tile (with a different identifier (i.e., the x,y image-tile location value)) moves into top-left corner of the view-plane grid (remember that you have to close & re-open the "View-plane list" box in order to see any changes since the table does not update automatically.).

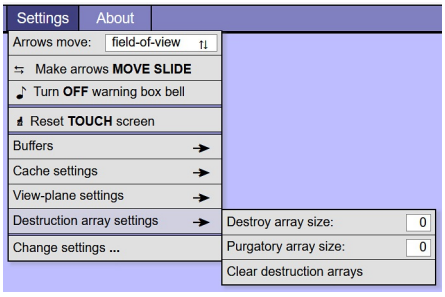


c. **"Show missing tiles ...".** Clicking on this button opens a movable box (the "**Missing Image Tiles**" box) which contains data that relate to the "wait-state" (described on page 8 (above, in section II.D.4.b.)) and to downloading image-tiles. In addition to the variables that identify each view-plane within the 'stack' ("index" (see V.C.3.b.(2). (above)), "F (focal plane)" (see V.C.3.b.(3). (above)), & "Z (zoom-level)" (see V.C.3.b.(4). (above))) or that indicate which view-plane "is visible" (see V.C.3.b.(1). (page 29, above)), the table in this box also shows which (if any) view-plane is causing the Viewer to be in a "wait state" (the "wait set" check-box) and the number of image-tiles that each view-plane is waiting to receive from the server (the "missing tiles" variable). This table is updated automatically every time an image-tile is received from the server or the visible zoom-level or focal plane is changed (this table behaves differently from the "cache list" table (see V.C.2.b (page 26, above)) or the "view-plane list" table (see V.C.3.b (page 28, above)), which are static snap-shots of the Viewer status at the time the button was clicked). Because it updates automatically, the "**Missing tiles**" table can be used to follow the downloading of image tiles from the server in real-time.

There are two buttons at the bottom of the "**Missing Image Tiles**" movable box. The "**Close**" button (at the bottom of the box) is equivalent to the "**X**" button in the top-right corner of the box; clicking on either of these buttons closes the box. There probably will never be a need to click on the "**Update**" button, because the data in the table is automatically updated. However, the automatic updating process only changes the parts of the table that have changed, and conceivably something might go awry in this piecemeal approach; clicking on the "**Update**" button causes the entire table to be rebuilt from 'scratch' (using the current status of the Viewer). For more about movable boxes, see VII. (page 34, below).

d. **"Remove excess focal planes".** It is unlikely that you will ever need to click on this button, since the Viewer automatically restricts the number of view-planes whenever the specimen is moved (see V.C.1.c. ("Maximum focal planes", page 23, above)). Clicking this button causes the Viewer to remove any view-planes that are not included by the settings for the "focal plane buffer", "zoom-level buffer", and "F×Z buffer" (see V.C.1 ("Buffers→", page 21, above)). This button ("Remove excess focal planes") is ***not*** displayed if the a single-focal-plane slide is being viewed, or if focusing has been disabled on a multifocal-plane slide (see V.A.4. ("'Disable focusing' button", page 19, above)).

4. **"Destruction array settings ➜".** Moving the mouse cursor over this tab (or touching the tab with your finger on a touch-screen device) causes a side-menu to be displayed that contains information and tools for manipulating the process that removes view-planes from the Viewer's 'stack' of view-planes. The Viewer normally handles removal and destruction of view-planes efficiently and automatically, and it is unlikely that you will ever have recourse to this side-menu (unless you are re-writing the computer code for the Viewer). However, these tools are available if something goes wrong.



When the Viewer changes magnification or (sometimes) focal planes, view-planes usually are removed from the Viewer's 'stack' of view-planes (see V.C.1 ("**Buffers➔**", page 21, above)). Several steps are required to "destroy" a view-plane after it is removed from the 'stack' of view-planes. Rather than making the user wait while the Viewer goes through all of the steps involve in destroying the discarded view-planes, these view-planes are removed from the 'stack' and dumped into a 'pile' (the destruction array) of view-planes-to-be-destroyed. These discarded view-planes are then destroyed in the background while the user studies the view-planes remaining in the 'stack'.

Under normal conditions, it is unlikely that discarded view-planes will accumulate in the destruction array (the 'pile' of view-planes waiting to be destroyed) because destruction of a view-plane is very fast compared to the user's actions. However, if something goes wrong (or if the user's computer is *very* slow), it is conceivable that view-planes waiting to be destroyed might accumulate in the destruction array. The items in this side menu ("**Destruction arrays ➔**") allow the user to identify and deal with this unlikely problem.

a. **"Destroy array size".** The value shown in this box is the number of view-planes awaiting destruction; it updates continuously (whenever a view-plane is added to the array or is destroyed). It is unlikely that you will see any value other than zero (normally, it probably takes less than a second to destroy 30 view-planes).

b. **"Purgatory array size".** This box shows the number of view-planes that have been removed from the 'stack' of available view-planes and are awaiting (in the "purgatory array") the decision (by the Viewer) whether to destroy these view-planes or to re-insert these view planes back into the 'stack' of view-planes. It is unlikely that you will see any number other than zero in this box.

If the user depresses the mouse button on the specimen (see II.D.2.a. ("**Using the mouse to move the slide**", page 4, above)), the Viewer assumes that the user is preparing to move the specimen and, if the Viewer's previous action had been focusing up-and-down, the Viewer removes any "extra" focal planes from the 'stack' of view-planes (see discussion in V.C.1.c. (page 23, above)). However, the user could change his/her mind, release the mouse button, and initiate another focusing operation without "moving" the specimen (i.e., "dragging" the specimen with the computer mouse). If this happens, the Viewer needs to reinstate the view-planes that it just removed from the 'stack' of view-planes. Because of this uncertainty, when the user depresses the mouse button on the specimen, the Viewer does ***not*** immediately move any "extra" focal planes from the 'stack' of view-planes into the destruction array (for more about the destruction array, see beginning of this section (V.C.4., above)); instead, the Viewer temporarily places these removed view-planes into a 'pile' of view-planes (the purgatory array) that is separate from the 'pile' view-planes that are being destroyed (the destruction array). If the user proceeds to move the specimen, the view-planes in this temporary 'pile' (purgatory array) are moved into the 'pile' of view-planes awaiting destruction (the destruction array) and are destroyed. If the user releases the mouse button without moving the specimen, the view-planes in the temporary 'pile' (the purgatory array) are re-inserted into the 'stack' of available view-planes.

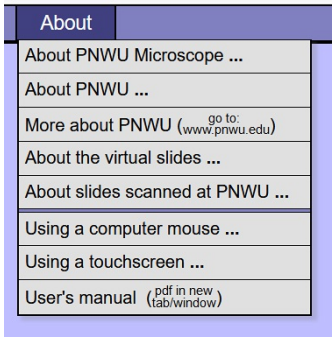
The Viewer also uses the purgatory array to temporarily hold the excess focal-planes removed from the 'stack' when the user touches the slide with *two* fingers (on a touch-screen device; see II.D.2.b. ("**Moving the slide with a touchscreen**", page 4, above)), because the same uncertainty exists (the user could raise his/her finger without moving the specimen) regardless of whether a mouse or a finger is used to "drag" the specimen. The purgatory array is *not* used when the user uses the navigator or menu to "move" the specimen (see II.D.2.c ("**Using the navigator or menu controls to move the slide**", page 4, above)). Since depressing the mouse button on the navigator's or menu's UP/DOWN/LEFT/RIGHT controls *immediately* initiates "movement" of the view-plane, there is no uncertainty with regards to the destiny of any "extra" view-planes when the navigator or menu controls are used, and in this case, these excess view-planes are transferred directly from the 'stack' to the destruction array.

c. **"Clear destruction arrays".** Clicking on this button forces the Viewer to destroy all view-planes in the destruction array before proceeding with any other user interactions (see beginning of this section, V.C.4. (above) for more about the destruction array). If there are any view-planes in the purgatory array, these also would be destroyed (however, there should not be view-planes in the purgatory array since clicking the mouse on a button (or touching a menu item with your finger on a touch-screen device) should clear the purgatory array (see V.C.4.b. (above))).

5. **"Change settings ..." (Introduction).** Many of the settings that control the performance of the Viewer have been "exteriorized" so that these variables can be adjusted when debugging the Viewer or to meet an individual user's unusual situation (e.g., an *exceptionally* slow computer, a computer with *very* limited memory, or an *exceptionally* slow internet connection). Clicking on the "**Change settings...**" button opens a box that allows the values of these variables to be changed. The default values chosen for these variables most likely are the optimal settings for your computer, and we ***strongly*** **discourage** you from tampering with these settings. If you encounter a problem with the Viewer, we encourage you to contact us (Microscope@PNWU.edu) *before* using the "**Change settings**" box to alter any of the Viewer's variables. If the "**Change settings**" box is open, you can close it by clicking on the box's "**Cancel**" button or by clicking on the "**X**" in the upper-right corner of the box.

The controls in the "**Change settings**" box are described in a separate section (see X. (page 41, below)).

VI. **"About" menu.** Moving the computer mouse over the "**About**" tab on the main menu (or touching the main menu's "**About**" tab with your finger (for a touchscreen device)) causes the "**About**" drop-down menu to appear below the main menu's "**About**" tab. The tabs (buttons) on this drop-down menu provide you with access to information and instructions regarding the PNWU Virtual Microscope. If the label on the menu's tab ends with an ellipsis ("..."), clicking on this button opens a movable box within the Viewer's window (see VII. (page 34, below) for more about movable boxes). As indicated in their labels, the other buttons (tabs) in the "**About**" drop-down contain links to other websites or documents (i.e., PDF files) which (depending the internet browser's settings) will be opened in a new window or in a new browser tab when the user clicks on the button.



A. **"About PNWU Microscope...".** Clicking on this tab opens a movable box that contains the copyright and license information for the PNWU Virtual Microscope. This box also contains email addresses that can be used to contact us.

B. **"About PNWU...".** Clicking on this tab opens a movable box that provides an introductory synopsis to College of Osteopathic Medicine at Pacific Northwest University of Health Sciences (PNWU), which is the institution that hosts the PNWU Virtual Microscope.

C. **"More about PNWU".** Clicking on this tab causes the internet browser to open a new window or tab (depending on the browser's settings) that links to the Pacific Northwest University of Health Science's website (https://www.pnwu.edu/inside-pnwu/about-us).

D. **"About the virtual slides...".** The movable box that opens when this tab is clicked contains information about the databases that are the sources for the virtual slides in the publicly-available version of the PNWU Virtual Microscope.

E. **"About slides scanned at PNWU…".** Clicking on this tab opens a movable box that discusses the sources of the "real" (glass) microscope slides that have been scanned at Pacific Northwest University of Health Sciences (PNWU). One of the goals of the virtual microscopy project at PNWU, in addition to building the PNWU Virtual Microscope, has been to create high-resolution multifocal-plane virtual microscope slides for teaching histology (initially, before we built the Viewer, we created high-resolution single-focal-plane virtual microscope slides). Since PNWU never used glass microscope slides for teaching histology, this project has depended on the generosity of others for the glass slides which we have been scanning, and the information in this box acknowledges our debt to the community of histologists for their assistance on this project.

The list of high-resolution virtual slides scanned at PNWU can be accessed from within the Viewer by clicking on the "Go to Slide Box" button (see II.C. ("**Choosing a slide**", page 2, above)) or from outside of the Viewer by using your internet browser to open the PNWU Virtual Slide Box (http://SlideBox.pnwu.edu), and then selecting "PNWU" from the "**Limit slides by: Source**" drop-down side-menu (and then clicking on the "**Get list of slides**" button). Instructions for using Slide Box can be found in Slide Box's "**About**" menu → "**Using SlideBox…**" option.

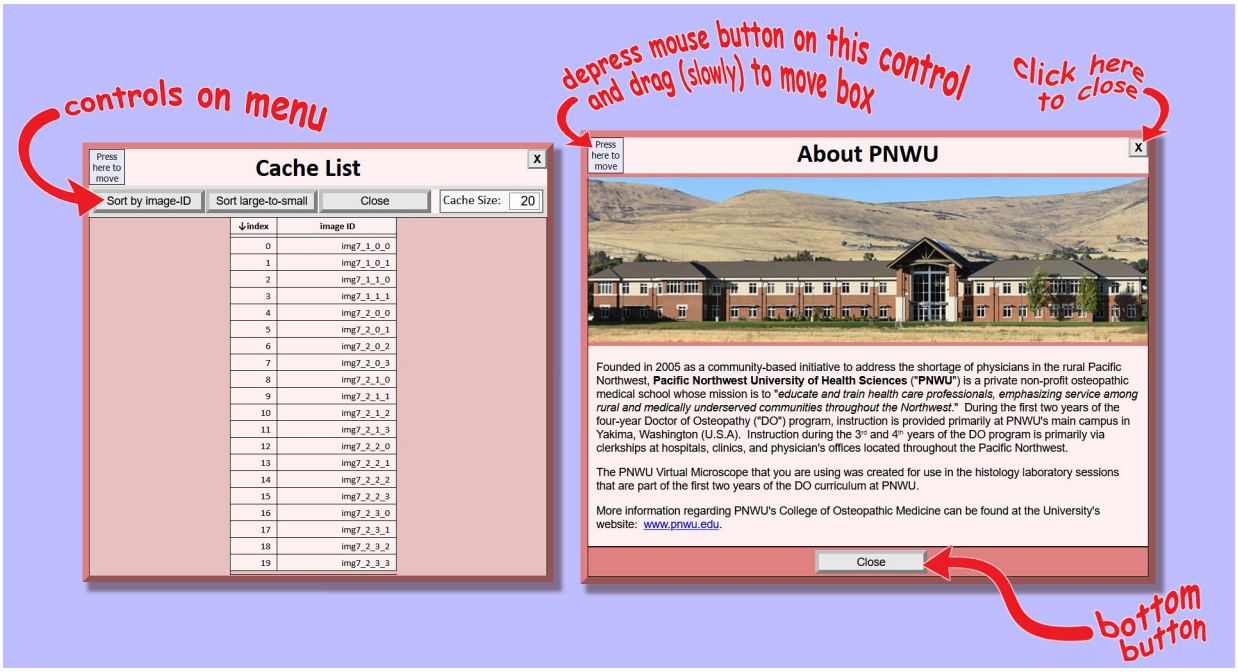
F. **"Using a computer mouse …".** The movable box that opens when this tab (button) is clicked contains a brief introductory synopsis of how to use a computer mouse to navigate around a virtual slide that is displayed by the Viewer. This synopsis is less complete, but more accessible, than the information provided in the "**Getting Started**" section of the PNWU Virtual Microscope Viewer User's Manual (this document, see II.D. (page 3, above)).

G. **"Using a touchscreen …".** The movable box that opens when this tab (button) is clicked contains a brief introductory synopsis of how to navigate around a virtual slide when the Viewer is being used on a touchscreen device. This synopsis is less complete, but more accessible, than the information provided in the "**Getting Started**" section of the PNWU Virtual Microscope Viewer User's Manual (this document, see II.D. (page 3, above)).

H. **"User's manual…".** Depending on your internet browser's settings, clicking on this button (the tab on the drop-down menu) either opens (in a new browser window or a new browser tab) or downloads the PDF file of the PNWU Virtual Microscope Viewer User's Manual (this document). The User Manual also can be accessed directly (http://viewer.pnwu.edu/docs/ViewerUserManual.pdf) by your internet browser from outside the Viewer.

VII. **Movable boxes.** When the Viewer needs to display more data than fits easily into a small box within a menu item, a separate box (technically, an HTML tag) containing the data is opened within the Viewer's window. The boxes that the Viewer uses for this purpose ("movable boxes") are all built around a common design, and (as the heading for this section implies) can be moved by the user to any location within the Viewer's window.

A. **Dragging the box.** All of the Viewer's movable boxes have a blue button (initially labeled "Press here to move") in the top-left corner of the box. Positioning the computer mouse over this blue button causes the mouse's cursor to change from the 'default' cursor icon (↖) to the 'move' cursor icon (✣). Depressing the mouse's button (while the mouse cursor is positioned over this button), or touching the blue button with your finger (for a touchscreen device), changes the button's text (from "Press here to move" to "Drag mouse to move"). Moving the computer mouse (while keeping the mouse button depressed) or your finger (for a touchscreen device) across the screen will drag the movable box across the screen. Movement of the "movable boxes" is a bit balky, and it works best if you drag these boxes *slowly*; if the mouse cursor moves off the blue box, *release* the mouse button, reposition the cursor over the blue box, depress the mouse button and restart dragging the box to its desired location.



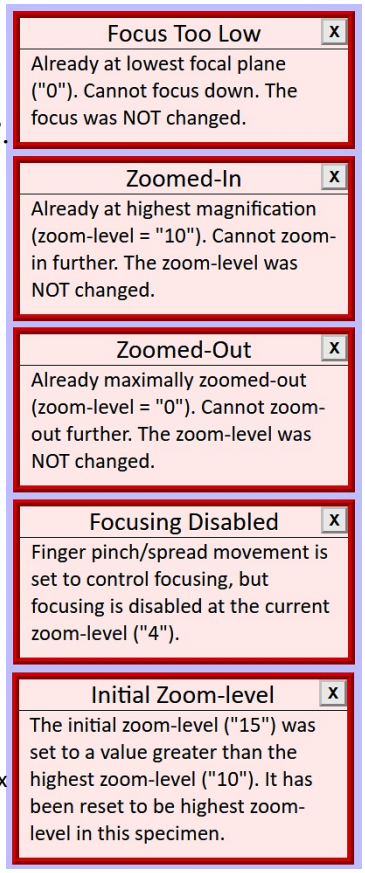
B. **Closing the box.** All of the Viewer's movable boxes have an "**X**" button in the upper-right corner of the box, and a "**Close**" button either in the box's menu (see VII.C. (below)) or at the bottom of the box (for the "**Go To**" box (see II.D.2.d. (page 5, above)), the "**Change Focus Settings**" box (see V.A.3. (page 19, above)), and the "**Change Settings**" box (see V.C.5. (page 33, above)), this button is labeled "**Cancel**", rather than "**Close**"). Clicking on either the "**X**" button or the "**Close**" button will close the movable box.

C. **Other box controls.** Depending on the nature of the data presented within the movable box, the movable box may have other, box-specific buttons. The number and location of these controls depends on the specific movable box, and some movable boxes only have the controls for moving (see VII.A. (above)) and closing(see VII.B. (above)) the box. If the movable box has box-specific controls, these controls are described in the sections of this manual that discuss the functions of the specific box.

   Depending on the specific movable box, the additional buttons may be located at the bottom of the box, or the box may have its own menu that contains these controls. If the movable box has its own menu, this menu is located between the box's heading (which contains the box's title, the blue "Press here to move" button (see VII.A. (above)), and the "**X**" button (see VII.B (above))) and the body of the box (which contains the box's data).

Although they contain different information, the movable boxes that can be opened by buttons (tabs) in the "**About**" drop-down menu (see VI. (page 33, above)) use the same object (HTML tag) to display their contents. As a result, if one of these "**About**"-menu movable boxes is open and you open another "**About**"-menu movable box, the Viewer must close the first movable box before it can re-use the HTML object for the second movable box. Thus, only one of the "**About**"-menu movable boxes can be open at a time. For instance, if the "**About PNWU**" box (see VI.B. (page 33, above)) is open and you click on the tab labeled "**Using a computer mouse...**" on the "**About**" drop-down menu (see VI.F. (page 34, above)), the "**About PNWU**" box will close and the "**PNWU Microscope with a Computer Mouse**" box will open. This limitation does not apply to the other movable boxes. For instance, the "**About PNWU**" box, the "**Missing Image Tiles**" box (see V.C.3.c. (page 31, above)), the "**View-plane List**" box (see V.C.3.b. (page 28, above)), and the "**Go To**" box (see II.D.2.d. (page 5, above)) could all be open at the same time.

VIII. **Warning boxes & User-response (error) boxes.** Many things can go wrong while the Viewer is attempting to display a slide. In some cases, where the user doesn't need to take any corrective action but should be made aware of the problem (so the user doesn't repeat the action that generated the error), the Viewer will transiently display a "warning box". Although the title and contents of the warning box depends on the error, all boxes of this class are similar in appearance and behavior.



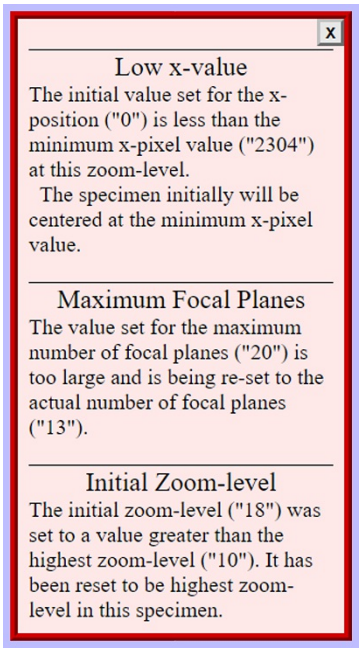
Warning boxes are transient, but their disappearance can be delayed. Normally, the warning box will fade away after about 1½ seconds (so the user doesn't need to do anything, except wait, to clear the box off the screen). However, the user can prevent this box from fading (in order to have more time to read the message) by moving the computer's mouse cursor over the warning box (or by touching the warning box on a touchscreen device). Fading of the box will resume when the mouse cursor (or the user's finger) moves off the warning box. Moving the mouse cursor over a partially-faded warning box (or touching the partially-faded box with your finger on a touchscreen devices) restores the box to its fully opaque (unfaded) state; however, once the box has completely faded, it is gone and moving the mouse over its former location will not recall it.

Clicking on the "**X**" button (in the upper-right corner of the warning box) causes the warning box to close immediately. With the default settings for warning box display & fade times, you probably will never need to use the "**X**" button; however, this button might be needed if you change the warning box settings.

The default display and fade times for the warning boxes are a compromise between readability (or time needed to use the mouse cursor to 'trap' the box in its unfaded state (see above)) and fading quickly (to avoid significantly interrupting the user). As a result, you probably should not change the settings that control this behavior. However, although ***not*** *recommended*, these settings can be changed (see X.J. (page 47, below)) using the "**Settings**" menu → "**Change settings...**" tab (see V.C.5. (page 33, above)).

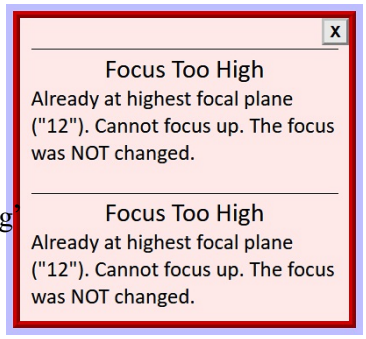
Warning boxes can include an audible alert. An audible alert can be associated with warning boxes, so that there is a "beep" ("toot") whenever the Viewer issues a warning. During beta-testing of the Viewer, most of the users found the "toot" to be annoying rather than helpful, so the default is to have the audible alert turned off. However, you can use "**Settings**" menu → "**Turn on warning box bell**" to turn the "toot" on (see V.B.3. (page 21, above)).

Warning boxes can include multiple warnings. If the Viewer generates a series of warning in a short period of time, rather than displaying multiple warning boxes (each of which would have to fade away before the next warning box could be displayed), the Viewer combines these warnings together into a single warning box. This sometimes occurs when there is an event, such as loading a slide, that requires that the Viewer reset a number of user-assigned settings.



warning boxes can include multiple warnings

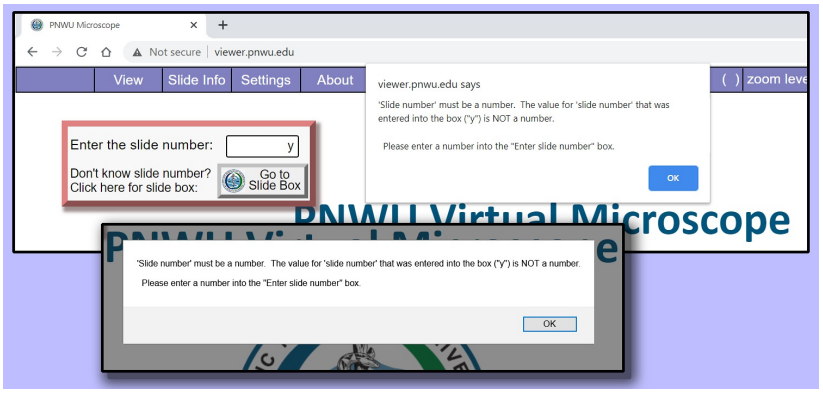
For instance, in the example above (previous page), the user had used the "**Change Settings**" box (see V.C.5. (page 33, above)) to set the value for the "maximum focal planes loaded" (see V.C.1.c. (page 23, above)), and then had used commands within the URL to set the initial x-position and zoom-level (see II.F.2. (page 15, above); the URL was: http://viewer.pnwu.edu?slide=3005&x=0&z=18).



Warning boxes can have several identical warnings. Repeating the same action will cause the Viewer to issue multiple identical warnings if the action generates a warning. If the action is repeated quickly (e.g., spinning the computer mouse's wheel), the Viewer will include these multiple (identical) warnings into a single warning box. This 'stuttering' is not a computer error, but rather a response to multiple instances of the same warning-generating action.

For instance, when using the computer mouse's wheel to change zoom-level or focal plane (see II.D.3.a. (page 6, above) & II.D.4.c. (page 9, above)), if the user continues to rotate the mouse's wheel after the zoom-level or focus limits have been reached, the Viewer will generate a series of warnings (one for each click (more-or-less) of the mouse wheel).

User-response (error) boxes. For some (usually important) warnings, the Viewer will display a warning and stop everything[13] until the user acknowledges the warning message by clicking on the appropriate button within the message-containing box. In these cases, the box that the Viewer uses to display the warning message is part of the browser's javascript interpreter (rather than a component that is unique to the PNWU Virtual Microscope); as a result, the appearance of these boxes will vary somewhat between browsers.



For instance, the illustration on the right shows the "user-response" box from two different browsers (Chrome & FireFox) to the same typographic error.

Hopefully, all of the "user-response" boxes that you encounter will be in response to issues such as typographic errors, and the text in the box should explain the issue and how to respond to it. However, the Viewer also will display a class of much more cryptic "user-response" error boxes if it encounters programming errors. If the Viewer ever gives you one of these more cryptic error messages, *please* write down the text of the message and email it to us (MicroscopeBugs@pnwu.edu; see I.B. ("**Talking to us**", page 1, above)), along with a description of what you & the Viewer were doing when the error message was generated.

[13] More technically, the execution of the Viewer's HTML/javascript code is suspended until the user clicks on a button ("OK", or either "OK" or "Cancel", depending on the warning/error message) within the "user-response box" (clicking one of these buttons causes the browser's built-in function (alert() or confirm()) to return control to the Viewer's javascript function that called the browser's built-in function).

IX. **"Command-line" arguments (the full list).** When invoking the Viewer (see II.A (page 1, above)), it is possible to include instructions to the Viewer ("command-line" arguments) within its URL; this was explained earlier in this document (see II.F ("**Command-line arguments (the short-version)**", page 13, above)). As noted in the earlier section, there only are a few of these "command-line" arguments that you probably will find useful. However, for completeness, the current section provides a list (in alphabetical order) of all of the command-line arguments available to the Viewer.

If command-line arguments are given, the server address (the main part of the URL: "http://viewer.pnwu.edu") *must* be separated from the command-line arguments by a question mark ("?"). Multiple command-line arguments can be included in the URL (after the "?"); these should be separated by ampersands ("&"). The command-line arguments are case-insensitive and white-space is ignored (for everything after the "?"; the first part of the URL has different rules). Thus, "... & Unmute Bell & ...", "... &UnmuteBell& ...", "... &unmutebell& ...", and "... &UNMUTEBELL& ..." are equivalent commands.

If there is a value (e.g., a number) associated with the command, this value *must* be separated from the command-name by an equal-sign ("... & CommandName = value & ..."). Some commands require a value, and for these commands, the value must be supplied as part of the command (within the URL; i.e., the Viewer will generate an error message rather than querying the user for a value if the required value is omitted). For commands that appear to be a statement (e.g., "... & Unmute Bell & ..."), providing a value is optional; if no value is provided the statement is treated as true. For these true/false commands, if a value is given, 1 is equivalent to true, and 0 is equivalent to false.

Thus, the commands: "... & Unmute Bell & ...", "... & Unmute Bell = true & ...", and "... & Unmute Bell = 1 & ..." are equivalent and would turn-on the "beep" that can accompany the warning box (see V.B.3. (page 21, above)). Conversely, "... & Unmute Bell = false & ..." or "... & Unmute Bell = 0 & ..." would turn-off the "beep" (however, since having the "beep" off is the default, this command would do nothing unless the user had already turned the "beep" on).

For the most part, the command-line arguments are read and implemented in their order (from left-to-right) within the URL. In most cases, this order is irrelevant, since most of these commands are independent of each other (this is ***not*** true for some "cs_*name* = value" commands). However, if conflicting commands are given within the URL (e.g., http://viewer.pnwu.edu?unMuteBell = true & unMuteBell = false), the last argument in the list usually will take precedence (i.e., in this example, the warning-box bell would be muted, since "unMuteBell = false" was the last command processed).

The available command-line arguments are listed (in alphabetical order) below:

1. value : This command is equivalent to "slide = value" (see IX.16 (page 41, below)). It sets the slide number to value (see II.C. (page 2, above); also see II.F.1. (page 14, above)). This command also prevents inclusion of the "**Chose a new slide**" tab in the main menu unless the "sb" command is also given (see IX.14 (page 41, below)).

2. arrowMoveSlide or arrMvSld : This command changes the initial setting for the direction that the specimen "moves" when the user depresses an UP, DOWN, LEFT, or RIGHT 'arrow' button on the navigator or "**View**" menu. If this command has been included in the URL, when the computer mouse's button is depressed while the mouse cursor is located over one of the 'arrow' movement buttons (or the user touches one of these buttons on a touchscreen device), the Viewer "moves" the slide (rather than the field-of-view) in the direction indicated by the arrows (see II.D.2.c. (page 4, above)). This command only determines the initial behavior of the 'arrow' buttons when the Viewer first loads; after the Viewer has loaded, the "**Settings**" menu → "Make arrows move slide/FOV" button still can be used to toggle whether the specimen or the field-of-view moves in the direction indicated by the arrows (see V.B.2. (page 20, above)).

This is a true/false command: "ArrowMoveSlide" is equivalent to "ArrowMoveSlide = true". Since the default in the absence of this command is for the field-of-view to move in the direction indicated by the arrow, the command "ArrowMoveSlide = false" normally has no effect.

3. cs_*name* = value : This command allows the user to use the command-line to change any of the Viewer's variables that can be adjusted using the "**Change Settings**" box (see V.C.5. (page 33, above)). This command is intended for use by computer programmers debugging revisions to the Viewer, and for adapting the Viewer to the specialized needs of users who have unusual network or computer restrictions. We ***strongly*** discourage you from using this command without first talking to us (to contact us, see I.B. (page 1, above)). For this command, *name* is the code (see X. (page 41, below)) identifying the variable that will be set to value.

4. f = value : This command sets the initial focal plane to value (see II.D.4. (page 7, above); also see II.F.2. (page 15, above)). If the initial zoom-level (see IX.20. (page 41, below)) is less than the "Zoom-level limit for focusing"(see V.A.1. (page 18, above)), then the "Default focal plane" also is set to value (see V.A.2. (page 18, above)).

5. F disable : This command disables the focusing controls (for a multifocal-plane specimen) when the slide is loaded. It has the same action as clicking the "**Setting**" menu → "Disable focusing" tab (see V.A.4 (page 19, above)), except it is implemented before the slide loads. After Viewer loads, this command can be reversed by clicking on the "**Setting**" menu → "Enable focusing" tab. The "Fdisable" command has no effect for single-focal-plane specimens. The command "Fdisable = true" is equivalent to "Fdisable" (without any value). The command "Fdisable = false" is formally equivalent to clicking on the "**Setting**" menu → "Enable focusing" tab; however, because the default is for focusing to be enabled, the command "Fdisable = false" normally has no effect.

6. hideF : This command causes the box displaying focal plane information to be omitted initially from the main menu (see II.E.7.c. (page 13, above; also, screen-image on page 8 (above, in II.D.4.)). The action of this command can be reversed (so the focal plane is displayed on the main menu) after the Viewer has loaded by clicking on the "**View**" menu → "Show **menu items**" → "Show **focal plane** on menu" tab (see III.A. (page 16, above)). This is a true/false command; "HideF" (without a value) is equivalent to "HideF = true". Including "HideF = false" in the command line prevents the Viewer from automatically removing the focal-plane box from the main menu if the main menu is too large to fit in the Viewer's window (for automatic 'trimming' of main-menu information boxes, see III.A. (page 16, above)).

7. hideLogo : This command terminates display of the box that normally is shown transiently as the Viewer is loading and that contains the program title and reference to the copyright & licensing information (the "Logo" box; see II.B. (page 2, above) & II.F.1. (page 14, above)). Display of this box, which normally fades in ~2½ seconds, is initiated before the Viewer processes the command-line arguments. Thus, the "Logo" box may be already displayed when the Viewer encounters the "hideLogo" command as it is reading the command-line arguments. When the Viewer reads the "hideLogo" command, the Viewer immediately hides the "Logo" box. As a result, the "Logo" box may briefly flash on the computer screen with the "hideLogo" command (although this will be much shorter than the box's fading in the absence of this command.). This command is a true/false command: "hideLogo" and "hideLogo = true" are equivalent commands. The command "hideLogo = false" normally has no effect since *not* hiding the "Logo" box is the default in the absence of this command.

8. hideName or hideNm or hideSlideName or hideSlideNm : This command causes the box showing the slide name to be omitted (initially) from the main menu (see II.E.2. (page 10, above)). After the Viewer has loaded, the action of this command can be reversed (so the slide name is displayed on the main menu) by clicking on the "**View**" menu → "Show **menu items**" → "Show **slide name** on menu" tab (see III.A. (page 16, above)). The command "HideName" (without a value) is equivalent to "HideName = true". Including "HideName = false" in the command line prevents the Viewer from automatically removing the slide-name box from the main menu if the main menu is too large to fit in the Viewer's window (for automatic 'trimming' of main-menu information boxes, see III.A. (page 16, above)).

9. hideNavigator or hideNav : This command causes the navigator (see II.D.1.a. (page 3, above)) to be hidden when the slide initially loads. After the slide loads, the user still can use the "**View**" menu → "Show **navigation controls**" button (tab) to turn-on display of the navigator (see III.B. (page 17, above)). This is a true/false command; the command "HideNav" (without a value) is equivalent to "HideNav = true". Since the default is for the navigator to be displayed (after a slide is loaded), "HideNav = false" (which would cause the navigator to be displayed) normally does nothing.

10. hideSlideName or hideSlideNm : same as "hideName" (see IX.8 (above)).

11. hideXY : This command hides the boxes, normally present on the main menu, that show the x,y-coordinates of the mouse cursor or (for a touchscreen device) of a single finger (see II.E.7.a. (page 11, above)). After the Viewer has loaded, the action of this command can be reversed (so the mouse's x,y-coordinates are shown on the main menu) by clicking on the "**View**" menu → "Show **menu items**" → "Show **x & y position** on menu" tab (see III.A. (page 16, above)). HideXY is a true/false command; the command "HideXY" (without a value) is equivalent to "HideXY = true". Including "HideXY = false" in the command line prevents the Viewer from automatically removing the x- and y-coordinate boxes from the main menu if the main menu is too large to fit in the Viewer's window (for automatic 'trimming' of main-menu information boxes, see III.A. (page 16, above)).

12. hideZ : This command causes the box displaying the zoom-level information to be omitted (initially) from the main menu (see II.E.7.b. (page 12, above)). The action of this command can be reversed (so the zoom-level is displayed on the main menu) after the Viewer has loaded by clicking on the "**View**" menu → "Show **menu items**" → "Show **zoom-level** on menu" tab (see III.A. (page 16, above)). HideZ is a true/false command; "HideZ" (without a value) is equivalent to "HideZ = true". Including "HideZ = false" in the command line prevents the Viewer from automatically removing the zoom-level box from the main menu if the main menu is too large to fit in the Viewer's window (for automatic 'trimming' of main-menu information boxes, see III.A. (page 16, above)).

13. muteBell : This is an obsolete command. "MuteBell = false" is equivalent to "unMuteBell" (see IX.17 (page 41, below)). Prior to September 2020, the default was to have an audible warning ("beep") associated with warning boxes (see VIII. (page 36, above)); in that situation,"MuteBell" turned-off the "beep". Once the default was changed to not have a "beep" accompany the warning boxes, including "muteBell" or "muteBell = true" in the URL does nothing (since the "beep" already is off). A "muteBell" command-line argument would cancel a the effect of any "unMuteBell" command preceeding it.

14. sb : This command forces the Viewer to include the "**Chose a new slide**" tab in the main menu (see II.C. (page 2, above)). In the absence of the "sb" command, specifying the slide number in the command-line (the "slide = value " command (see IX.16 (below)) or the "value " command (see IX.1 (page 38, above))) suppresses display of the "**Chose a new slide**" tab in the main menu (see II.F.1 (page 14, above)). The "sb" command overrides this suppression, allowing the user to specify a slide in the command-line but also allowing the user to select another slide later without having to invoke a new instance of the Viewer.

15. sb = value : This command sets slide number to value (see II.C. (page 2, above); also see II.F.1. (page 14, above)), ***and*** forces the Viewer to include the "**Chose a new slide**" tab in the main menu (see II.C. (page 2, above)). Thus, this command combines the "sb" command (see IX.14 (above)) with the "slide = value " (see IX.16 (below)).

16. slide = value : This command sets the slide number to value (see II.C. (page 2, above); also see II.F.1. (page 14, above)). This command also prevents inclusion of the "**Chose a new slide**" tab in the main menu unless the "sb" command is also given (see IX.14 (above)).

17. unMuteBell : This command causes an audible warning ("beep"/"toot") to sound whenever a warning box is displayed (see VIII. (page 36, above)). This command-line argument only determines the Viewer's initial behavior; the user still can toggle the warning bell on/off using the "**Settings**" menu → "Turn **ON/OFF** warning bell" tab (button). This is a true/false command: "unMuteBell" is equivalent to "unMutebell = true". Normally, "unMuteBell = false" has no effect, since the default is for the warning bell to be muted.

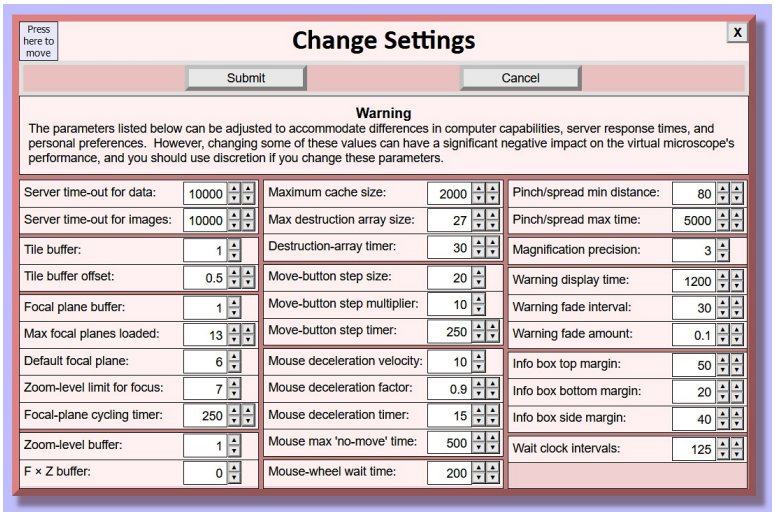
18. x = value : This command adjusts the slide's initial horizontal position so that the x-coordinate of the center of the Viewer's window is value (see II.E.7.a. (page11, above); also see II.F.2. (page 15, above); value is in specimen-pixels (see footnote 5 (page 12, above))).

19. y = value : This command adjusts the slide's initial vertical position so that the y-coordinate of the center of the Viewer's window is value (see II.E.7.a. (page11, above); also see II.F.2. (page 15, above); value is in specimen-pixels (see footnote 5 (page 12, above))).

20. z = value : This command sets the initial zoom-level to value (see II.D.3. (page 5, above); also see II.F.2. (page 15, above)).

X. **Changing settings (full list).** The parameters that control much of the behavior of the Viewer have been exteriorized, so that they can be accessed when debugging the Viewer and so that the Viewer can be adapted to unusual operating conditions (e.g. computers with very limited memory or very slow network connections). The default values for these variables should be optimal for most users, and *you should talk to us **before** changing these settings* (for contact information, see I.B. (page 1, above)).

The exteriorized parameters can be accessed either by clicking on the "Change settings ..." tab (button) on the "**Settings**" drop-down menu after the Viewer has loaded (see V.C.5. (page 33, above)), or by including "cs_*name* = value " command-line arguments in the Viewer's URL when the Viewer is invoked (see IX.3. (page 39, above)). Clicking on the "**Settings**" menu → "Change settings ..." tab opens a the "**Change Settings**" movable box (for more about movable boxes, see VII. (page 34, above)). Within the "**Change Settings**" box, each parameter has a text-edit box, into which the user can type a value, and 'spinner' buttons on which the user can click to change the value. When the "**Change Settings**" box opens, parameters that have their default value are shown in black, and any parameters which have been changed from their default values are shown in blue.



When a setting is changed using the "**Change Settings**" box, the new value is ***not*** effective until the user clicks the "Submit" button on the box's menu (clicking the "Submit" button also closes the "**Change Settings**" box). New parameter values that have not yet been submitted are shown in red (unless the new value is the default value (shown in black) or the current parameter value (shown in blue if different from the default value). The values for multiple parameters can be changed before clicking the "Submit" or "Cancel" button. If the user clicks the "Cancel" button on the box's menu, the Viewer's settings are *not* changed (and the "**Change Settings**" box is closed without saving any values that had been entered into the box).

For most of the parameters, their value must be a non-negative integer, although the values for a few parameters are floating-point numbers.

Below is a brief explanation of each of the parameters whose values can be changed using the "**Change Settings**" box (in the order that these parameters are listed in the box). These parameters also can be changed using "cs_*name* = value" command-line argument (see IX.3 (page 39, above)). In the explanations given below, the text-string that corresponds to "*name*" in "cs_*name* = value" for each parameter given in parentheses following the name of the parameter.

A. **Timer settings.**

1. **Server timeout for data** (cs_SQLTimOut)**:** This is the interval (in milliseconds) between the Viewer submitting a request (for data) to the PNWU Virtual Microscope's SQL server and the Viewer generating a timeout error (if the data have not been received). When a timeout error occurs, the Viewer gives the user the option of waiting another interval (or ending the session, since the slide cannot be viewed if the SQL server is unresponsive).

2. **Server timeout for images** (cs_WaitTimOut)**:** This is the interval (in milliseconds) between when the Viewer enters a "wait state" (because it is waiting for image-tiles from the PNWU Virtual Microscope database server) and when the Viewer generates a time-out error (because it is still waiting for image-tiles; "wait states" are described on page 8 (above, in II.D.4.b. (page 8, above)). When a timeout error occurs, the Viewer gives the user the option of waiting another interval or displaying the view-plane with missing image tiles.

   This timeout interval is for the wait state; it is *not* a timeout for the request to the server. The user usually is unaware of delays in the server's response to image-tile requests because the view-planes are buffered and the image-tiles normally are loaded in the background (image-tile and view-plane buffering are described in V.C.1. (page 21, above)).

B. **X,Y-buffer settings.**

1. **Tile buffer** (cs_XYBuf)**:** This integer specifies the number of image-tiles that form a buffer on each side of each view-planes (see V.C.1.a. (page 22, above)).
2. **Tile buffer offset** (cs_XYOff)**:** This floating-point number specifies the fractional amount of a buffer tile that must be exposed on the computer screen (i.e., is no longer a buffer tile) before triggering the Viewer to add new buffer image-tiles (to the buffer-tile-deficient side of the view-plane; this also triggers removal of image-tiles from the opposite side (which now has too many buffer tiles) of the view-plane).

C. **Focal plane settings.**

1. **Focal plane buffer** (cs_FBuf)**:** This integer specifies the number of focal planes on each side of the currently-visible view-plane that are loaded into the view-plane 'stack' to serve as a buffer (when focus is changed after a non-focusing operation; see V.C.1.b. (page 22, above)).
2. **Max focal planes loaded** (cs_MaxF)**:** This integer specifies the maximum number of focal-planes that can be loaded into the view-plane 'stack' during a sequence of focusing operations (see V.C.1.c. (page 23, above)).
3. **Default focal plane** (cs_DefF)**:** This parameter was discussed in a previous section (see V.A.3.a. (page 19, above)) in the context of the "**Change Focus Settings**" box (this parameter is included in the "**Change Focus Settings**" box because it might need to be changed during normal use).
4. **Zoom-level limit for focus** (cs_ZFLim)**:** This parameter was discussed in a previous section (see V.A.3.b. (page 19, above)) in the context of the "**Change Focus Settings**" box (this parameter is included in the "**Change Focus Settings**" box because it might need to be changed during normal use).
5. **Focal-plane cycling timer** (cs_FTimr)**:** This parameter was discussed in a previous section (see V.A.3.c. (page 19, above)) in the context of the "**Change Focus Settings**" box (this parameter is included in the "**Change Focus Settings**" box because it might need to be changed during normal use).

D. **Zoom-level settings.**

1. **Zoom-level buffer** (cs_ZBuf)**:** This integer specifies the number of zoom-levels on each side of the currently-visible view-plane that are loaded into the view-plane 'stack' to serve as a buffer (see V.C.1.b. (page 22, above)).
2. **F × Z buffer** (cs_FZBuf)**:** The value of this parameter should be 0 and should ***not*** be changed. This integer specifies the number of off-axis view-planes (i.e., neither the focal plane nor zoom-level match the currently-visible view-plane) on each side of the currently-visible view-plane that are loaded into the view-plane 'stack' (see V.C.1.e. (page 25, above)).

E. **Cache & destruction array settings.**

1. **Maximum cache size** (cs_MxCache)**:** This integer sets the maximum size of the image-tile cache (see V.C.2.d. (page 26, above)).
2. **Max destruction array size** (cs_MxDstArr)**:** This integer sets the maximum size of the destruction array (see V.C.4.a. (page 32, above)). If the size of this array exceeds this value, the Viewer will notify the user and pause operation while it destroys the view-planes that have accumulated in this array.

   It is unlikely that the Viewer will ever need to pause because of a full destruction array. If the Viewer pauses frequently to empty this array, it would be best for you to contact us (see I.B. (page 1, above)) before adjusting the maximum size of the destruction array, since the value for this parameter is a trade-off between the number of view-planes 'dumped' into the destruction array at the end of a focusing operation and the need to quickly recycle image-tiles (from the discarded view-planes) into the cache.

If the destruction array timer interval (see X.E.3. (below)) is set to a large value (do ***not*** do this!), it may be necessary to decrease the maximum destruction array size to ensure that the Viewer does not need to reload image-tiles from a discarded view-plane before the view-plane is destroyed.

3. **Destruction-array timer** (cs_DstArrTimr)**:** This integer is the interval (the 'break' that the Viewer takes; in milliseconds) between destruction of view-planes. The Viewer cannot respond to user commands while it is in the middle of destroying a view-plane. To retain responsiveness to the user while it is removing view-planes in the destruction array (see V.C.4. (page 31, above) for an explanation of the destruction array), each time the Viewer destroys a view-plane, it normally 'takes a short break' before destroying the next view-plane. During this 'break', the Viewer handles any actions requested by the user.

   The length of this interval has to be kept short to ensure that the destruction array is emptied efficiently, but it also needs to be long enough to catch any user actions.

F. **Move-button settings.** To understand the parameters that are described here (section X.F.), it may be helpful to refer to the earlier part of this document that described the behavior of the UP/DOWN/LEFT/RIGHT 'arrow' buttons on the navigator and on the "**View**" menu (see II.D.2.c. (page 4, above)).

1. **Move-button step size** (cs_MvBtnStp)**:** This integer is the distance (in screen-pixels; see footnote 5 (page 12, above)) that the slide normally "moves" each time it takes a 'step' when the 'arrow' tabs (buttons) on the "**View**" drop-down menu or the single-arrow buttons on the navigator are depressed.

   Large values for this parameter can be disorienting because the specimen jumps so far in a 'step' that the user loses visual cues as to the previous and current location (so the amount that the specimen has moved becomes confused). Conversely, small values for this parameter can be frustrating because it takes the Viewer multiple 'steps' (and, therefore, a long time) to move the slide the desired distance.

2. **Move-button step multiplier** (cs_MvBtnMult)**:** This number (an integer > 1) is the amount by which the 'step'-size (see X.F.1. (above)) is multiplied if the <SHIFT> key also is depressed when an 'arrow' button on the navigator or "**View**" menu is depressed. In addition, the step-size for the navigator's outer "triple-arrow" buttons is the value of "Move-button step size" (see X.F.1. (above)) multiplied by this value ("Move-button step multiplier").

   Setting this parameter to a large value can be disorienting (because the 'step' is so large that the user loses visual cues as to location) and can cause the Viewer to over-step its x,y-buffer (see V.C.1.a. (page 22, above)), resulting in the view-plane initially having missing image-tiles (these "empty" tiles will be replaced as the image-tiles are received from the server). Conversely, setting this parameter to too small a value decreases the utility of the navigator's "triple-arrow" buttons and of using the <SHIFT> key in conjunction with 'arrow' movement buttons.

3. **Move-button step timer** (cs_MvBtnTimr)**:** This integer is the interval (in milliseconds) between 'steps' when the Viewer is "moving" the specimen in response to the user depressing one of the 'arrow' buttons on the navigator or on the "**View**" menu.

   The length of this interval needs to be long enough that the user can react (i.e. release the 'arrow' button) to one 'step' of the specimen before the next 'step' occurs. Conversely, the interval needs to be short enough that the user doesn't get impatient waiting for the next 'step'.

G. **Slide deceleration settings.** When a mouse (see II.D.2.a. (page 4, above)) or two fingers (for a touchscreen device; see II.D.2.b. (page 4, above)) is/are used to "move" the specimen, the rate at which the slide decelerates after the user 'releases' the slide is determined by the parameters listed in this section.

1. **Mouse deceleration velocity** (cs_MusMxDeclVel)**:** This integer is maximum value for the initial deceleration velocity (in pixels/millisecond). Setting a maximum velocity was necessary to prevent calculation of unreasonably large velocities (or divide-by-zero errors) when the movement interval was very short.

   Small values for the "Mouse deceleration velocity" parameter can result in the slide suddenly slowing down when the mouse button is released (or one of the primary fingers is lifted off a touchscreen). Large values for this parameter can result in the slide initially increasing in speed when the mouse button is released (or one of the primary fingers is lifted off a touchscreen).

2. **Mouse deceleration factor** (cs_MusDeclMult)**:** This number (which must be between 0.10 and 0.98) is multiplied by the current velocity of the slide to determine the slide's new velocity for the next iteration of the slide deceleration process.

   The "Mouse deceleration factor" interacts with the "Mouse deceleration timer" (see X.G.3. (below)) to determine the rate at which the slide slows down when the user releases the slide from a slide-movement operation. Small values of "Mouse deceleration factor" will cause the slide to slow down very quickly (as though there was a lot of friction acting on the slide), while large values of this parameter will allow the slide to "glide" for a long time before coming to a full stop.

3. **Mouse deceleration timer** (cs_MusDeclTimr)**:** This integer is the interval (in milliseconds) between each iteration ('step') of the slow-down-movement process during the slide's deceleration after the slide is released from a mouse or touch movement operation.

   This "Mouse deceleration timer" interval has to be short enough so that the deceleration appears to be a smooth action (rather than a series of discrete steps, each with a smaller step-size than the previous step).

4. **Mouse max 'no move' time** (cs_MusMxPause)**:** This integer is the length of time (in milli-seconds) that the slide must *not* move while the mouse button is down (or two fingers are touching the slide) in order for the specimen to "stay at rest" when the mouse button is released (or a finger is lifted off the touchscreen).

   The initial velocity (for the slide-deceleration process) when the mouse button is released normally is the average velocity of the slide over the previous nine "mousemove events".[14] If the slide has been "held" stationary for a noticeable period of time, it is disconcerting for the slide to start moving when the mouse (or finger) is released (the slide will start to move because the slide's average velocity over the previous nine mousemove events is non-zero). To prevent this re-initiation of slide movement, if the time between the last mousemove event and release of the mouse's button is greater than the value of the "Mouse max 'no move' time" parameter, the slide remains stationary when the mouse button is released (or a finger is lifted off the touchscreen).

H. **Mouse-wheel & pinch/spread settings.**

1. **Mouse-wheel wait time** (cs_MusWhlWait)**:** This integer is the refractory period (in milli-seconds) following a mouse-wheel-induced change in magnification (see II.D.3.a. (page 6, above)) or focus (see II.D.4.c. (page 9, above)); during this refractory interval, the Viewer ignores signals from the mouse wheel. The "Mouse-wheel wait time" parameter also controls a similar refractory period during pinching/spreading finger actions on a touchscreen device (see II.D.3.b. (page 6, above) & II.D.4.d (page 9, above)).

[14] A "mousemove event" is an interrupt (an "event" in javascript jargon) that is sent to the Viewer by the internet browser whenever the computer mouse moves over the Viewer's window. When the mouse appears to drag the slide across the screen, the Viewer actually is changing the location of the currently-visible view-plane (relative to the top-left corner of the Viewer's window) every time it receives a mousemove event from the browser.

The user can 'spin' the computer mouse's wheel faster than the user can respond to the changes caused by rotating the mouse wheel. The "Mouse-wheel wait time" interval gives the user time to react to the change in magnification or focus, decreasing the likelihood of the Viewer overshooting the user's intended action (and circumventing very long strings of warning messages (see VIII. (page 36, above)) when the Viewer reaches the top or bottom zoom-level or focal plane).

Very small values for the "Mouse-wheel wait time" parameter most likely will result the user thinking that the mouse-wheel is 'too sensitive', while very large values will make the mouse-wheel seem slow or unresponsive.

2. **Pinch/spread min distance** (cs_PnchDist)**:** This integer is the amount that the distance (in screen-pixels (see footnote 5 (page 12, above))) between the two fingers on a touchscreen device must change in order to generate a pinch (decrease in magnification or focal plane) or spread (increase in magnification or focal plane) action.

   On a touchscreen device, two fingers touching the specimen are used to move the slide (see II.D.2.b. (page 4, above)) and to change either magnification (zoom-level; see II.D.3.b. (page 6, above)) or focus (focal plane; see II.D.4.d. (page 9, above)). Since we don't want a slight jitter or tremor to cause the Viewer to change magnification or focus, we require that changes in zoom-level or focal plane only occur if the the fingers pinch together or spread apart by a significant amount (the number of pixels set by the "Pinch/spread min distance" parameter).

   Small values for the "Pinch/spread min distance" parameter will make the touchscreen more responsive to pinching or spreading finger-actions, but also increase the chance of an unwanted zoom-level or focal plane change and makes the pinch/spread bug (see II.D.3.b. (page 6, above)) more obvious and annoying. Large values for the "Pinch/spread min distance" parameter will make the touchscreen seem balky and unresponsive to pinching or spreading actions.

3. **Pinch/spread max time** (cs_PnchWait)**:** This integer is the time interval (in milliseconds) that the Viewer uses when determining whether the distance between the two fingers (that are touching the specimen on a touchscreen) has changed sufficiently to constitute a "pinch" or "spread" action (see X.H.2. ("Pinch/spread min distance", above)).

   When using a touchscreen device, the user's fingers can remain on the touchscreen for a substantial time and, during that long time interval, there may be a slight drift in the distance between the two fingers that the user does not intend to be a pinch or spread action. By limiting the length of time that it "looks" backwards when calculating whether a pinch or spread occurred, the Viewer eliminates unintentional finger drift and requires that the pinch or spread be an intentional, relatively rapid, action by the user.

   The optimal setting for the "Pinch/spread max time" parameter should depend on the user's behavior. If the user tends to move his/her fingers quickly, then a smaller value should be better. If the user moves his/her fingers slowly and deliberately, then a larger value should be better. By trial-and-error, an interval of about 5 seconds seems to work well, but performance seems to be relatively insensitive to this parameter over a fairly wide range of reasonable values.

   Whenever a "touchmove event"[15] occurs on the part of the Viewer's window that contains the specimen, the Viewer enters the locations of the two fingers that are touching the specimen into a 'table' (an array of javascript objects) and discards any entries in the 'table' that are older than the value of the "Pinch/spread max time" parameter. The Viewer looks 'backwards in time' through this 'table' to see if the difference between the current distance-between-fingers and any previous distance-between-fingers is great enough to constitute a "pinch" or "spread".

[15] A "touchmove event" is an interrupt (an "event" in javascript jargon) that is sent to the Viewer by the internet browser whenever a finger touching the Viewer's window (on a touchscreen device) is moved. The Viewer uses the data (a "TouchList" object) that it receives as part of this "touchmove event" to determine what action (if any) needs to be performed.

I. **Floating-point number precision.** If a decimal number (a "floating-point number") is the result of a division operation, the resulting number can have many non-zero digits after the decimal point (and possibly non-significant digits before the decimal point) . In these cases, the Viewer may need to round-off some of these digits in order to properly display the number. For some variables, the user has the option to specify the precision (number of digits) with which the number is displayed.

- **Magnification precision** (cs_MagPrec)**:** This integer determines the total number of digits (excluding place-holding zero's before the decimal point) that are included in the "Magnification" and "Max magnification" values displayed on the "**Slide Info**" drop-down menu (see II.E.7.b. (page 12, above)).

J. **Warning box settings.** The parameters in this section control the transient appearance and fading of warning boxes (see VIII. (page 36, above)).

1. **Warning display time** (cs_WarnDispTime)**:** This integer is the amount of time (in milliseconds) that the warning box normally is displayed before it starts to fade.
2. **Warning fade interval** (cs_WarnFadeTimr)**:** This integer is the interval (in milliseconds) between each 'step' as the warning box fades.

   This parameter ("warning fade interval") should be set to a value that is small enough so the 'steps' of the fading process are not perceived by the user and the warning box appears to fade away smoothly. If you want to prolong the fading process, it probably is better to decrease "warning fade amount" (see X.J.3. (below)), rather than increasing "warning fade interval".
3. **Warning fade amount** (cs_WarnFadeAmt)**:** This floating-point number (which must be between 0.01 and 0.50) is the amount that the opacity[16] warning box decreases at each 'step' of the fading process.

The time (in milliseconds) required for a warning box to fade is:

fading-time = "warning fade interval" / "warning fade amount".

Thus, the total time (in milliseconds) that a warning box is on the screen (so it can be 'trapped' by moving the mouse cursor over the box (see VIII. (page 36, above))) is:

total-time = "warning display time" + ("warning fade interval" / "warning fade amount").

[16] Opacity is a property that can be assigned to elements (HTML tags) on a webpage. If opacity = 1 for a box on a webpage, then the box is opaque and any parts of the webpage that are located behind the box are hidden; conversely, if the box's opacity = 0, the box would be transparent. Initially (before fading commences at the end of the "warning display time" interval), the warning box's opacity = 1.

K. **Movable box settings.** The Viewer's first movable information-boxes (see VII. (page 34, above)) were created early in the development of the PNWU Virtual Microscope Viewer, and the design of these boxes evolved as development of the Viewer proceeded. As a result, not all movable boxes behave identically (updating the behavior of the 'early' movable boxes is on our "to do" list). For some of these boxes (for instance the "**Change Settings**" box; see beginning of this main section (section X., page 41, above)), the box's format is adapted to the size of the Viewer's window. The parameters listed (below) in this section control the formatting of some boxes and the initial location of all movable boxes (these boxes can be "dragged" to a new location within the Viewer's window after the box is open (see VII.A. (page 34, above))).

1. **Info box top margin** (cs_InfoBoxTop)**:** This integer is the distance (in screen-pixels) between the top of the Viewer's window and the top of a movable box when the movable box is initially displayed. This parameter determines the initial vertical location of all movable boxes.

2. **Info box bottom margin** (cs_InfoBoxBottom)**:** For movable boxes that adapt to the size of the Viewer's window, this integer is the minimum distance (in screen-pixels) between the bottom of the movable box and the bottom of the Viewer's window.

   This parameter ("Info box bottom margin") has no effect on movable boxes that do not reformat to fit the size of the Viewer's window, with the result that these boxes can extend below (and be hidden by) the bottom of the Viewer's window (the size of the Viewer's window will vary from user to user; it is determined by the size of the internet browser's window, which usually is determined by the size of the user's computer screen). In some cases, it may be possible to fit these non-adapting boxes within the Viewer's window by decreasing the value for the "Info box top margin" parameter (see X.K.1. (above)).

3. **Info box side margin** (cs_InfoBoxSide)**:** For movable boxes that adapt to the size of the Viewer's window, this integer is the minimum distance (in screen-pixels) between the left and right sides of the movable box and the left and right sides of the Viewer's window, respectively. This parameter ("Info box side margin") has no effect on movable boxes that do not reformat to fit the size of the Viewer's window.

L. **Wait-clock icon.** For touchscreen devices that do not have a mouse cursor, the existence of a wait-state is indicated by a spinning clock-face icon (; wait-states are described on page 8 (above; in section II.D.4.b. (page 8, above))).[17] Currently, only one of this icon's parameters can be changed via the "**Change Settings**" box (other parameters affecting this wait-icon may be exteriorized in the future).

- **Wait clock intervals** (cs_WaitClkTimr)**:** This parameter determines how fast the arm of the "wait-clock" icon "rotates" around the clock-face. There are eight images of the clock-face (with the clock's arm in different locations), and this integer is the time interval (in milliseconds) that each image of the clock-face is displayed before the icon cycles to the next clock-face image.

  The value chosen for the "wait clock intervals" parameter should be small enough so that the arm of the clock appears to sweep smoothly around the circumference of the clock-face. The time for a complete rotation of the clock's arm is approximately:

  rotation time (in milliseconds) = 8 × "wait clock intervals"

[17] The spinning clock-face icon is not displayed continuously during a wait-state. This icon is displayed only if the Viewer is in a wait-state, ***and*** the user touches (on a touchscreen device) the specimen with two fingers, or touches buttons (tabs) on the navigator or on a drop-down menu that would move or change the view-plane 'stack' if the Viewer was not in a wait-state.

XI. **Abbreviations for stains.** The list given below is limited to stains whose names that are so abbreviated by the Viewer that the abbreviation probably requires an explanation. This list is ***not*** a complete list of all stains used to provide contrast to specimens in the PNWU Virtual Microscope database.

For reasons of space, the Viewer sometimes uses cryptic abbreviations for the names of stains. Some of these abbreviations (e.g. "H&E" or "PAS") are widely-used and will be familiar to most users of the Viewer. However, even widely-used abbreviations (such as "H&E") can be so cryptic as to be unintelligible to students just beginning their study of histology. The Viewer also utilizes abbreviations of stain names that are less common or may be more-or-less unique to the Viewer, and these abbreviations require elucidation for a more general audience. Given below (in alphabetical order) are abbreviations for stain names that are used by the Viewer where the abbreviation seems to be particularly cryptic, or where there is the possiblity of confusion with a different stain (e.g. in the PNWU Virtual Microscope, "Masson" (without additional text) refers to Masson trichrome stain, rather than Masson's hematoxylin or Masson HES (hematoxylin, erythrosine, saffron) stain, and "Mallory" (without additional text) refers to Mallory trichrome stain, rather than Mallory's alum hematoxylin or Mallory's PTAH (phosphotungstic acid-hematein) stain).

Naming of stains can be very confusing and ambiguous. In some cases, very similar (or sometimes identical) staining procedures may have different names, while in other cases similar stains, or sometimes not-so-similar stains (for instance, the "green" and "blue" versions of Masson's trichrome stain, and variants of these versions), are lumped together under the same name. In addition, distinctly different stains sometimes have very similar names (often because they carry the name of a scientist who invented more than one staining protocol, for instance, "Weigert's iron hematoxylin" and "Weigert's iron resorcin fuchsin"). We have tried to make the Viewer's naming of stains as accurate and consistent/rational as possible, but there undoubtedly is room for improvement. Please do not hesitate to contact us (Microscope@PNWU.edu) if you feel that the name applied to a stain is incorrect, or if you believe that the stain used for a specimen has been misidentified.

- H&E ➜ hematoxylin (alum hematoxylin) & eosin.
- IHAB ➜ iron hematoxylin & aniline blue.
- Mallory ➜ Mallory trichrome stain.
- Mallory azan ➜ Heidenhain's azan modification of the Mallory trichrome stain.
- Masson (blue) ➜ Masson trichrome stain utilizing aniline blue as the fiber stain.
- Masson (green) ➜ Masson trichrome stain using Light green SF yellowish or Fast Green FCF as the fiber stain.
- PAS ➜ periodic acid Schiff reaction, or periodic acid & Schiff's reagent.
- Tol.blue ➜ Toluidine blue.