STEPS FOR USING THE PIXCELL II AND SUCCESSFUL LCM

SYSTEM STARTUP

A). TURN ON POWER STRIP, TO POWER UP THE ENTIRE SYSTEM AND THE DELL PC's MONITOR.

B.) TURN ON DELL COMPUTER.

C.) TURN ON THE OLYMPUS MERCURY ARC LAMP BURNER, **ONLY** IF USING FLUORESCENCE (TURN ON 20 MINS **BEFORE** PEFORMING LCM)

PREPARING FOR AN LCM SESSION

D.) PULL THE CAP CASSETTE OUT OF THE DOVETAIL ASSEMBLY AND LOAD YOUR CONSUMABLE OF CHOICE (EITHER CAPSURE OR CAPSURE HS).

E.) ONCE LOADED, INSERT THE CASSETTE BACK INTO THE DOVETAIL.

F.) POSITION THE JOYSTICK APPROXIMATELY PERPENDICULAR TO THE TABLETOP. THIS IS THE COARSE OPTICAL ALIGNMENT OF THE CAP.

G.) USING YOUR FINGERS, POSITION YOUR SLIDE OVER THE OBJECTIVE (4X) AND, USING THE OCULAR, LOCATE YOUR WORK AREA. FOCUS THE STAGE AS NEEDED. THE IDEAL WORK AREA SHOULD HAVE OPEN SPACE FOR TEST FIRING THE LASER AND CELLS OF INTEREST TO DISSECT.

H.) ACTUATE THE "VACUUM" CHUCK TO HOLD THE SLIDE IN PLACE ON THE STAGE. THE VACUUM IS PROVIDED BY A VACUUM HOSE CONNECTING THE LASER CONTROLLER TO THE "H-GROOVE" ON THE MICROSCOPE STAGE. THE GROOVE SHOULD ALWAYS BE COVERED WITH THE LEFT PORTION OF THE MICROSCOPE SLIDE. THE VACUUM IS ACTIVATED BY PUSHING THE 'VACUUM' BUTTON ON THE FRONT OF THE LASER CONTROLLER.

I.) ADJUST THE STAGE FOCUS AS NEEDED. AT THIS POINT, THE WORK AREA IS CHOSEN BY MOVING THE JOYSTICK.

CONDUCTING AN LCM SESSION

J.) PUSH/PULL THE CAP CASSETTE UNTIL A NEW CAP IS POSITIONED NEXT TO THE "LOAD" LINE ON THE STAGE. WITHOUT LIFTING THE ARM, SWING THE CAPPING ARM COUNTER-CLOCKWISE UNTIL IT STOPS IN POSITION OVER THE NEW CAP.

K.) RAISE THE CAPPING ARM TO DETACH THE NEW CAP AND SLOWLY MOVE IT FULLY CLOCKWISE UNTIL IT STOPS, AND THEN LOWER IT ONTO THE TISSUE SECTION.

L.) TO VISUALIZE THE COLORS OF THE TISSUE SECTION, DEPRESS THE SHUTTER CABLE BUTTON LOCATED ON THE JOYSTICK. ADJUST (INCREASE) THE MICROSCOPE WHITE LIGHT LEVEL (SLIDE CONTROL ON THE FRONT LEFT OF THE MICROSCOPE BASE).

M.) WHEN READY TO USE THE LASER, DEPRESS THE SMALL LEVER BUTTON IMMEDIATELY UNDERNEATH THE SHUTTER CABLE BUTTON TO RETRACT THE

VISUALIZER. LOWER THE WHITE LIGHT LEVEL AS NEEDED. SELECT THE 10X OBJECTIVE BY ROTATING THE OBJECTIVE TURRET.

N.) ACTUATE THE LASER BY TURNING THE KEY ON (1/4-turn CLOCKWISE). WHEN THE UPPER DISPLAY ON THE LASER CONTROLLER IS SCROLLING A MESSAGE, THE LASER INTERLOCK IS BEING CHECKED FOR CONTINUITY. ONCE COMPLETED, THE LASER IS TURNED ON BY PRESSING THE "ENABLE" BUTTON GIVING A GREEN LED INDICATION.

O.) MOVE THE SPOT SIZE SELECTION LEVER (LEFT HAND SIDE OF LASER TOWER) TO THE SMALL SPOT SIZE POSITION (FULLY BACK).

P.) WHILE WATCHING THE LASER BEAM ON THE SONY AND STILL ON THE 10X OBJECTIVE, ADJUST THE LASER FOCUSING KNOB (BLACK KNOB UNDER THE SPOT SIZE LEVER) BACK OR FORTH UNTIL A SHARP LASER SPOT IS OBSERVED ON THE MONITOR.

Q.) TEST FIRE THE LASER AND OBSERVE THE PRESENCE OF A BLACK, UNIFORMLY THICK CIRCLE OF MELTED PLASTIC ON THE CAP. OBSERVATION OF THE BLACK RING(S) IS VISUAL CONFIRMATION THAT THE LASER IS FOCUSED PROPERLY, AND THE PARAMETERS CHOSEN ON THE LASER CONTROLLER (POWER and DURATION) ARE ADEQUATE FOR THE TISSUE SECTION UNDER EXAMINATION. IF NO BLACK RINGS ARE OBSERVED THEN LCM WILL NOT OCCUR. DO NOT PROCEED UNTIL PROPER MELTING IS VERIFIED.

R.) ONCE PROPER MELTING IS VERIFIED, YOU ARE READY TO DISSECT. POSITION YOUR CELLS UNDER THE LASER. KNOW THAT IN THIS SYSTEM, THE LASER IS STATIONARY. DISSECTION OCCURS BY MOVING YOUR CELL(S) UNDER THE LASER LIGHT (SPOT). FIRE THE LASER ONTO YOUR CELL(S). FIRE UNTIL ALL DESIRED CELLS HAVE BEEN "SHOT".

S.) WHEN SATISFIED THAT ENOUGH CELLS HAVE BEEN COLLECTED, RAISE THE CAPPING ARM, MOVE IT TO THE REST POSITION, AND OBSERVE THE HOLES LEFT BEHIND IN THE TISSUE.

T.) TO OBSERVE THE CAPTURED MATERIAL, RELEASE THE VACUUM, MOVE THE SLIDE TO THE LEFT UNTIL CLEAR GLASS IS OVER THE OBJECTIVE'S VIEW, REAPPLY THE VACUUM, AND RETURN THE CAPPING ARM TO THE WORK POSITION. OBSERVE THE CAPTURED CELLS USING THE OCULAR AND THE SONY. APPLY THE VISUALIZER IF COLOR RENDITION IS NEEDED.

U.) IF NO ADDITIONAL CELLS NEED TO BE COLLECTED, THEN MOVE THE ARM TO THE CAPPING STATION TO DROP OFF THE CAP (FULLY UP AND FULLY COUNTERCLOCKWISE, THEN LOWER INTO THE STATION). SWING THE ARM AWAY (CLOCKWISE WITHOUT LIFTING) TO EXPOSE THE COMPLETED CAP. USE THE CAPPING TOOL TO TRANSFER THE CAP (WITH TISSUE!) INTO THE EPPENDORF TUBE.

V.) ANALYZE THE TUBES' CONTENTS AS DESIRED.

W.) RETURN TO THE OFFICE AND AWAIT WORD OF YOUR NOBEL PRIZE.

USE OF THE WORKSTATION

X.) THE PIXCELL II IS NOT A PC-CONTROLLED INSTRUMENT. CELLS CAN BE CAPTURED WITHOUT USE OF THE PC. THE WORKSTATION IS USED WHEN THERE IS A NEED TO CAPTURE IMAGES FROM YOUR LCM SESSION. TO START THE ARCTURUS SOFTWARE, DOUBLE-CLICK THE ARCTURUS ICON ON THE DESKTOP.

Y.) PROCEED THROUGH THE DATA ENTRY MENUS UNTIL THE 'LIVE VIDEO' FEATURE IS REACHED. AT THIS POINT THE LASER CAN BE CONTROLLED VIA THE TOOLBAR AND IMAGES CAN BE CAPTURED.

Z.) IMAGES CAPTURED FALL INTO 4 CATEGORIES. 'ROADMAPS' ARE TYPICALLY LOW MAGNIFICATION VIEWS OF LARGE AREAS OF INTERESTS. 'BEFORE SHOTS' ARE NORMALLY HIGHER (10X or 20X) MAGINIFICATION VIEWS OF THE WORK AREAS. 'AFTER SHOTS' ARE IMAGES OF THE 'HOLES' LEFT IN THE TISSUE AFTER LCM IS COMPLETED AND THE CAP MOVED AWAY. AND FINALLY, THE 'CAP SHOTS' ARE IMAGES TAKEN OF THE CAPTURED MATERIAL ON THE CAP's FILM.

AA.) CAPTURED IMAGES CAN BE ANNOTATED AND MASSAGED WHILE SEATED AT THE WORKSTATION.

BB.) CAPTURED IMAGES CAN BE REVIEWED USING THE 'REVIEW DATA' OPTION OF THE SOFTWARE.

CC.) CAPTURED IMAGES CAN BE TRANSFERRED TO OTHER LOCATIONS FOR ADDITIONAL TORTURING, OFTEN WITH PHOTOSHOP, BY COPYING TO A ZIP DISC.

DD.) TYPICALLY, CAPTURED IMAGES ARE COMPRESSED TO JPG SIZE UPON EXITING THE SOFTWARE, ALTHOUGH THEY CAN BE KEPT AS TIF FILES AS WELL. A TIF IS ~1 Mb, WHEREAS A JPG IS ~300Kb.

USE OF THE FLUORESCENCE ATTACHMENT

DD.) THE MERCURY ARC LAMP POWER SUPPLY SITS ADJACENT TO THE LASER CONTROLLER. TURN IT ON AND ALLOW AT LEAST 20 MIN. WARMUP TIME.

EE.) THE FLUORSECENCE ATTACHMENT HAS 4 POSITIONS, AND THREE 'COLOR' CUBES. THE POSITIONS ARE: BRIGHT FIELD (the pass through port for the white light containing no cube), BLUE, GREEN, AND ORANGE. THE POSITIONS ARE SELECTED BY ROTATING THE CUBE TURRET UNDER THE STAGE.

GG.) THE TURRET HAS A SHUTTER BUILT IN. IT SHOULD BE OPEN WHEN USING A CUBE, BUT IN THE CLOSED POSITION WHEN NOT USING THE ATTACHMENT.

HH.) THE LIGHT PATH OF THE FLUORESCENCE ATTACHMENT HAS AN APERTURE WHICH IS LEVER ADJUSTED (using the lever underneath the stage). THE POSITION ON THE SCREEN IS ADJUSTED USING THE 'STICKS' ON THE LEFT HAND SIDE OF THE SCOPE, UNDER THE STAGE.

II.) SIGNAL AVERAGING IS POSSIBLE IN ORDER TO ENHANCE THE SIGNAL INTENSITY AVAILABLE FROM WEAKLY EMITTING SAMPLES. ON THE CAMERA CONTROL BOX, THE NORMAL POSITION IS USED FOR ROUTINE OPERATION (ie, no fluorsecence or very strong fluorescence). FOR WEAK EMITTERS, INCREASE THE INTEGRATION TIME CONSTANT FROM 'NORMAL' TO WHATEVER SETTING IS NEEDED FOR SUCCESSFUL VIEWING. THE WHITE LIGHT SETTING SHOULD BE VERY LOW.

JJ.) WHEN FINISHED, THE CUBE TURRET SHOULD BE 'PARKED' IN THE BRIGHT FIELD POSITION (no cube in the light path) AND THE TURRET SHUTTER SHOULD BE CLOSED.

KK.) TURN MERCURY LAMP POWER SUPPLY OFF.

END OF SESSION SHUTDOWN

LL.) FOR CONSISTENCY WHEN THE USER GROUP IS LARGE, SHUT THE PIXCELL DOWN IN THE SAME MANNER DAILY. IF THE PC HAS BEEN IN USE, SHUT IT DOWN USING THE SHUTDOWN BUTTON (that has the Microsoft "flag" on it).

MM.) ONCE THE PC HAS SHUTDOWN, TURN THE DELL MONITOR OFF USING THE POWER SWITCH.

NN.) PLACE THE PIXCELL CAPPING ARM IN THE REST POSITION AND RELEASE THE VISUALIZER BY DEPRESSING ITS BUTTON.

OO.) TURN OFF THE VACUUM.

PP.) TURN OFF THE LASER AND TURN OFF THE KEY (1/4-turn counterclockwise).

QQ.) TURN OFF THE POWER STRIP.

RR.) REMOVE CONSUMABLES AND COVER MICROSCOPE.