Guide to using the Knife Edge Scanning Microscope (KESM 2.0)

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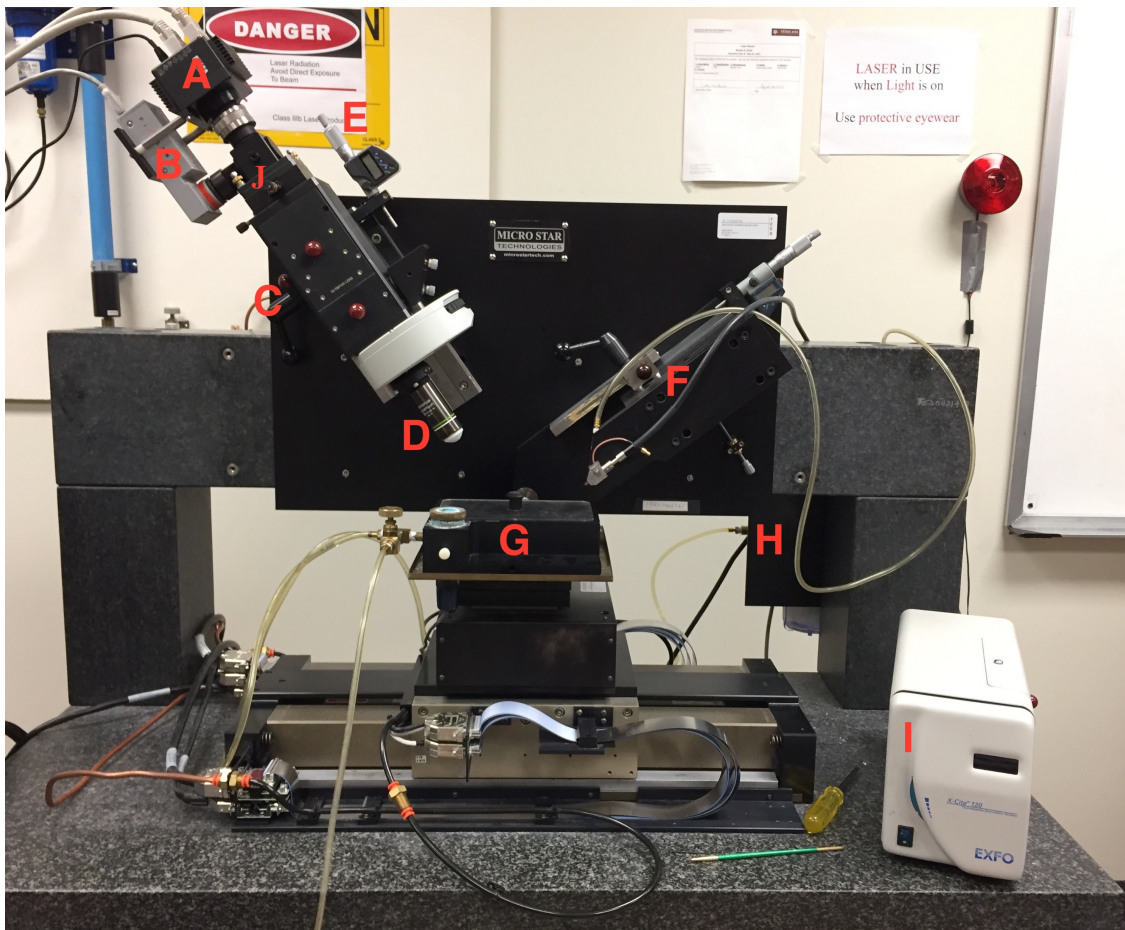July 2017

1   KESM process

Figure 1: KESM machine components: A) Line scan camera, B) Side camera, C) Latch keeping objective arm up, D) Objective, E) Knob adjusting objective location, F) Latch keeping knife arm up, G) stage where specimen resides, H) pump switch location, I) illuminator, J) Knobs adjusting camera location.

1. Check cable connections (camera, stage controller)

2. Check air pressure to stage

3. Turn on the Stage Controller (AEROTECH)

4. Make sure knife and objective arms are UP (see Fig.1)

1.1   First time setup

1. Use U500mmi program to get positional data for input into stage controller

   (a) load KESM parameters (at mmi/KESM/KESM.prm)

   (b) reset U500mmi (if "Invalid firmware" error happens, load firmware at U500/mmi/U500pci.jwp)

   (c) make sure stage is centered

   (d) enable X, Y, and Z axes

   (e) home Z, Y, and X (in order). Make sure the tubes are not stuck.

   (f) change speed to units per second by entering 'pr me un/se' or 'program metric units/second' in the command prompt

   (g) select jog

   (h) change speed for X, Y, and Z to 20, 5, and 1 respectively. Mode(Index). Distance(per move).

   (i) move to position X=155, Y=22 (roughly centering)

   (j) lower the knife arm and turn on the Illuminator (lowest setting)

   (k) find the bounding box extents of the tissue cube with respect to the knife, namely the (X,Y) position of the corners, and the depth of the height of the block (Z) (see Fig. 2)

      - This can be done by jogging the stage to each position and recording the FEEDBACK position
      - A quicker approach can be used by disabling a single axis (X or Y only) and repositioning by hand, but be aware this makes ALL BUT THE FEEDBACK DATA INVALID
      - For acquiring the Z position one can position the knife over the stage and move the stage up in increments of 0.1 distance until close (if holding the light above the knife you should see a shadow come into focus below on the tissue), then switching to 0.01 for potential collision. If the to much distance is used in the Z direction the knife can be dislocated in its the housing.
      - Another approach for the Z axis is to disable the X axis and move the Z axis up while manually 'slicing' across the block until a collision occurs (DO NOT USE MUCH FORCE WHEN PUSHING THE KNIFE OVER THE TISSUE)

   (l) close the U500mmi program, raise knife arm, and turn down the Illuminator dial when finished

2. Turn on the Camera (see Fig.1)

3. Turn on the Illuminator (with dial off or lowest initially) if not on already

4. Start the Stage Controller 2 application for a New Block (e.g. KESM(H:) / kesm2 / StageController / StageController.exe).

   (a) Create a new project (date automatically provided, but list details of what you are cutting) (Fig. 3-A)

   (b) Set the Stage Parameter Settings (Fig. 3-C)

      - Make sure speed doesn't go above 21 (average 19 + deviation 2). If the speed is too slow (e.g. below 10), the camera buffer will not be filled.
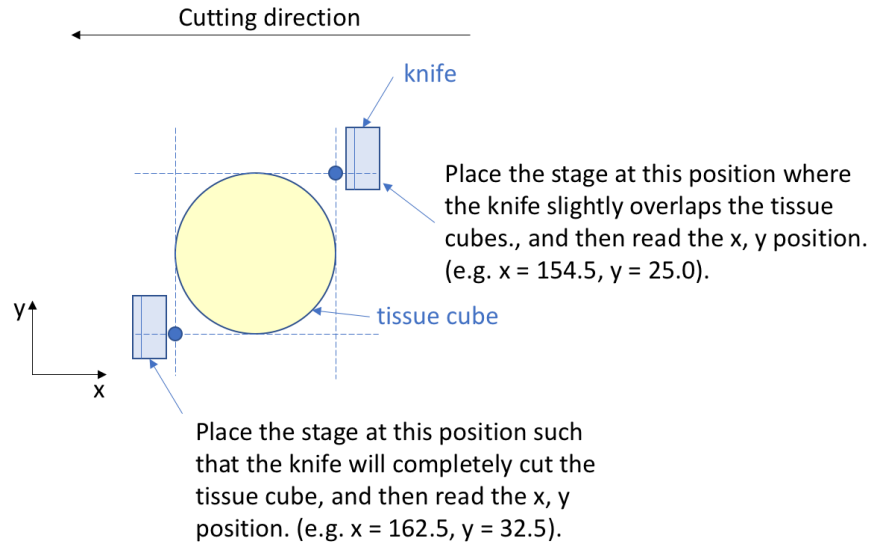
Figure 2: Find bounding box of the tissue cube. Empirically, it is convenient to find the x positions first, then the y positions, and the z position. See text for details.

- Slice Length should be your X width + 10 (due to camera buffer not loading first part when stage signals)
- Block width should be your Y width from your measurements
- Slice thickness should be 0.001 (1 um)
- Column Width should be 1.5 mm. The column width can be adjusted according to an objective field of view.
- Cutting Edge should be Left

(c) Make sure knife and objective arms are UP (see Fig.1)

(d) Make sure stage is centered (will not hit knife or objective when homing) (see Fig.1)

(e) Click the Init button (Fig. 3-E) and select Yes (stage will home and go to a default position). Wait until the initial position numbers show up in the GUI (e.g. about 155 for X) (Fig. 3-H).

(f) Select the Step by Step button (Fig. 3-F) (stage will move and software will generate a CurState file)

(g) Close the Stage Controller 2 application

(h) Go to the session folder and modify the CurState file (e.g. H: / kesm2 / Data / Project-name / CurState.txt) to be your parameters (X,Y,Z starting position in the 1st line), (stair step position in the 2nd line - DOESN'T MATTER), (Z height for the stage for each column → set to be your Z initial). NOTE your X starting position should be your least X - 8.5 as there is an issue with the stage's initial signals not causing the camera buffer to fill.

1.2 Subsequent setup

1. Use Stage Controller 2 for an existing project

(a) Start stage controller 2

(b) Open project (Fig. 3-B)

(c) Make sure knife and objective arms are UP (see Fig.1)

3

(d) Make sure stage is centered (see Fig.1)

(e) Run Init (Fig. 3-E) and select Yes (should stop at CurState home parameters). Wait until the numbers show up (Fig. 3-H).
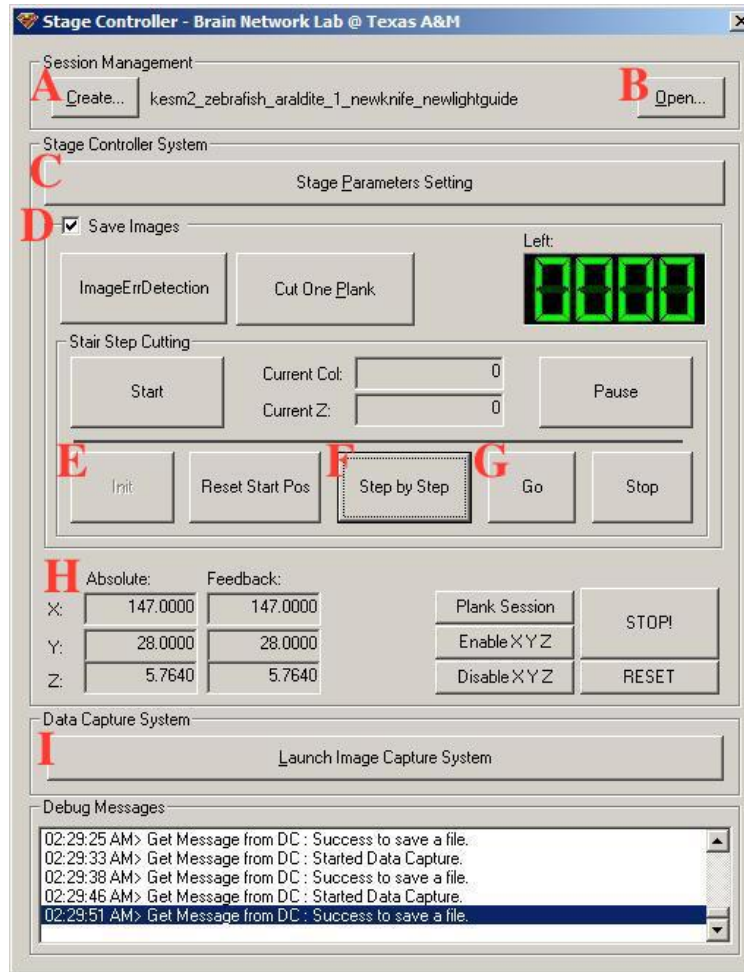

Figure 3: KESM Stage Controller GUI

1.3 Cutting specimen

1. Lower arms for knife and objective

2. Lock the knife in position tightly

3. Start the pump (should prime it to avoid overheating due to air in pump). Make sure the water level in the stage is enough.

4. Camera Focusing

   (a) Camera focusing with the side camera (PixeLink camera).
      i. Set the illuminator to lowest setting.

ii. Start the PC (Acer) in which the PixeLink camera application is installed.

iii. Launch Capture OEM.

iv. Set the preview size to 1280 x 1024 (Fig. 4(a)-A).

v. Run Video preview → Play (Fig. 4(a)-B).

vi. Adjust focusing with the objective knob (Fig. 1-E) to get the knife edge (blade) focused in the side camera preview as in Fig. 4(b).
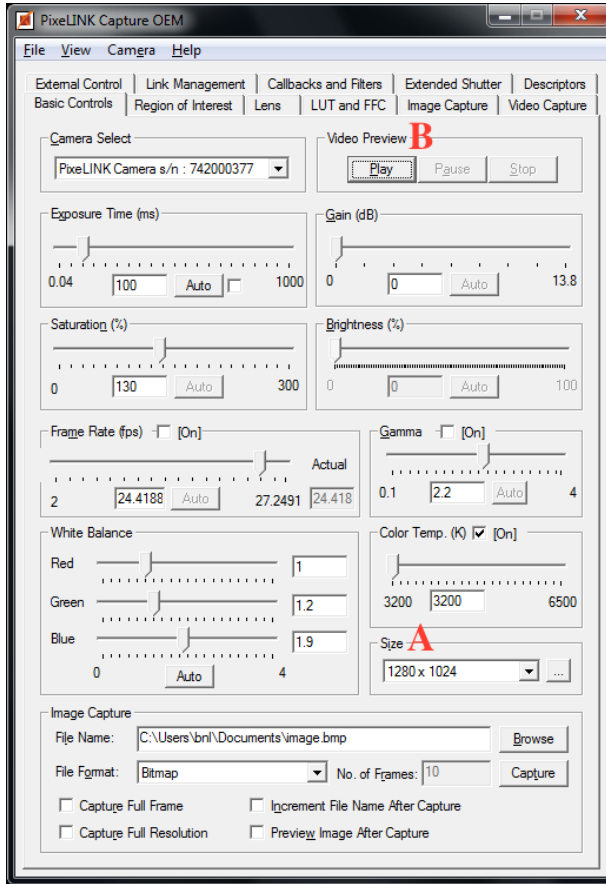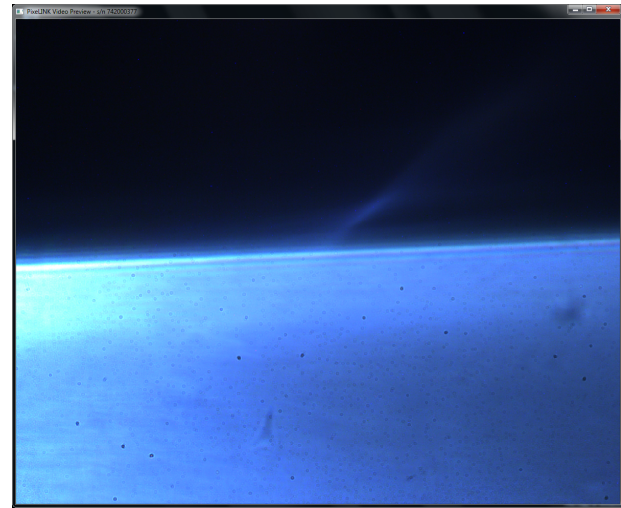
(a) PixeLink captureOEM   (b) Video streaming

Figure 4: **Focusing with the PixeLink camera application.** (a) PixeLink camera OEM application. (b) Video streaming displaying the knife blade. See text for details.

(b) Camera focusing with the line scan camera (DALSA).

i. Close the Data Capture window (Fig. 8) and launch Sapera CamExpert to focus the line scan camera.

ii. Select the configuration file for camera focusing (at ../ sapera_v.8.02 / camFiles / User / D_P2-4X-02K40_(Piranha_2)_8-bits_Default.ccf)

iii. Click Stretch Display (Fig. 5-B).

iv. Click Statistics (Fig. 5-C) to launch the statistics window. Select Line Profile in the statistics window (Fig. 6-A).

v. Click Grab/Freeze (Fig. 5-A) to start streaming.

vi. Adjusting focusing with the objective knob (Fig. 1-E) and the camera knobs (Fig. 1-J) such that:
- The boundary in Fig. 5-D should be a sharp vertical line between the dark and the bright area.
- Fine horizontal lines should be seen and the light is evenly distributed in Fig. 5-E.
- The Line Profile graph shows even distribution of the light intensity across the knife as in the Fig. 6-B.

vii. Close the Sapera CamExpert and launch the Data Capture GUI by clicking Image Capture System (Fig. 3-I). Select the configuration file for data capturing (at ../ sapera_v.8.02 / camFiles / User / D_P2-4X-02K40_(Piranha_2)_8-bits_external_sig.ccf) in the configuration window (Fig. 7).
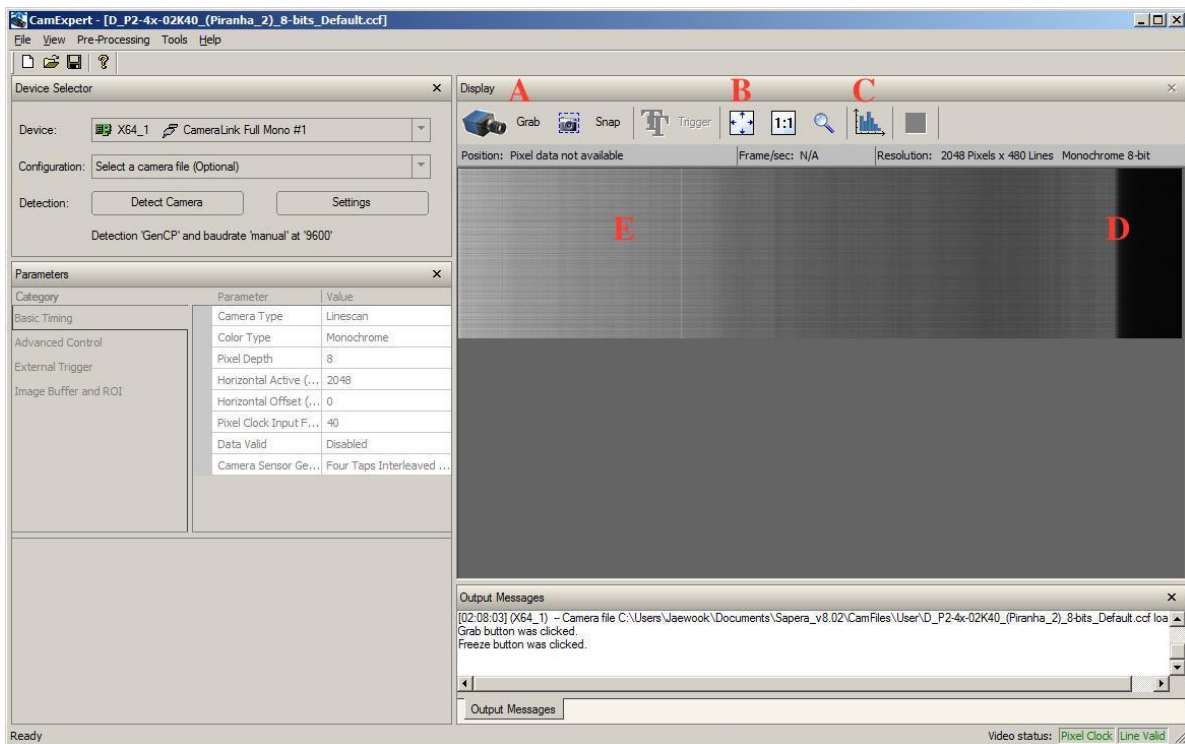

Figure 5: CamExpert. (A) Grab/Freeze button to start or stop the camera streaming. (B) Stretch Display button. (C) Launch statistics window button. (D) Displaying the line scan image of the boundary of the knife corner (i.e. the bottom corner of the knife blade in Fig. 2). When focused, the boundary should be a sharp vertical line between the dark and the bright area. (E) Displaying the line scan image of the knife blade. When focused, fine horizontal lines should be seen and the light is evenly distributed in this area.

5. Make sure Save Images box is checked (Fig. 3-D).

6. Select Step by Step button (Fig. 3-F) to cut a single slice (likely won't cut tissue yet)

7. Go to image in the Data Capture GUI (Fig. 8) and double check focusing (perhaps going back to the Sapera CamExpert steps)

8. Proceed to Go (Fig. 3-G) and allow slices to be cut until issue arises or no one is able to monitor (to ensure large portions of data not lost check every 30 minutes the lighting and focus)

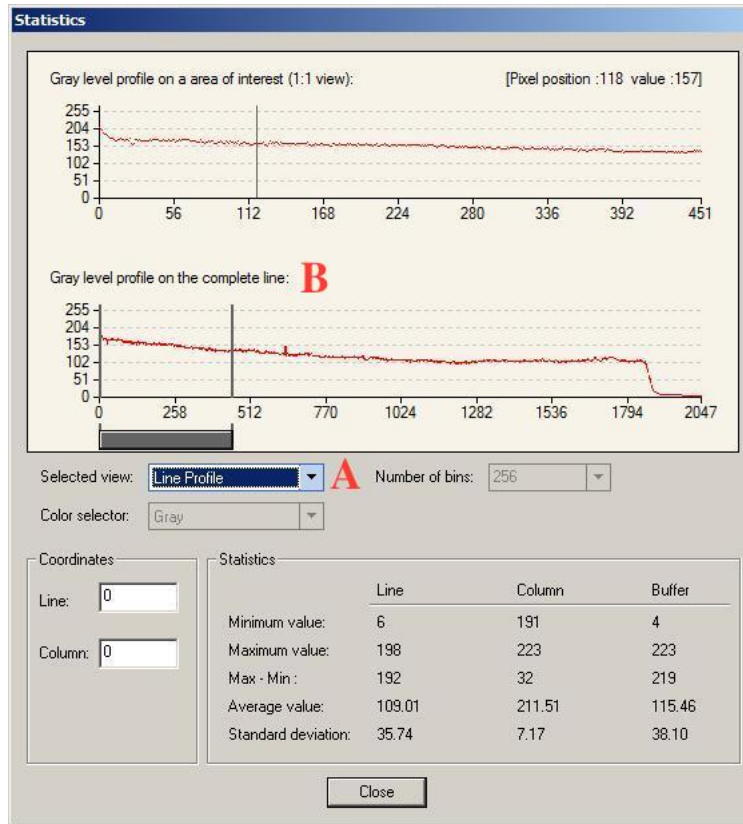9. Run the 'return to stair step' button (Fig. 3-G) to stop (may take 1-2 slices after button pressed).

Figure 6: CamExpert statistics window. (A) Select view of the graph. Line Profile selected. (B) The graph shows a selected view. When focused, the Line Profile graph shows even distribution of the light intensity across the knife as in the figure.

1.4 When stopping

1. Turn off the illuminator (see Fig.1)

2. Cut off the power to the pump (see Fig.1)

3. Cut off the power to the camera (see Fig.1)

4. Raise knife and objective arms UP (see Fig.1)

5. Place cover over stage (prevent things from falling into it)

6. If finished for the day, you can leave the stage power on (allows for resuming cutting with less setup). So that you do not need to Run "Init" again in the stage controller GUI for resuming cutting.

7. If finished for the week (weekend) turn the stage power off

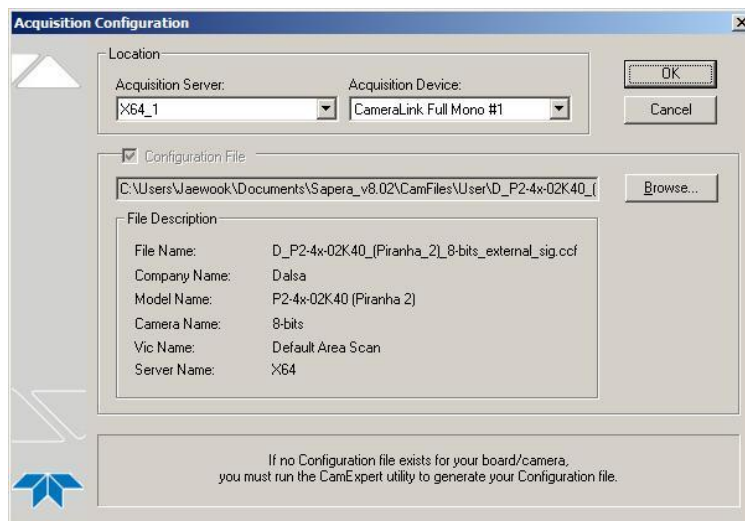8. Backup data to storage server (e.g. one of the NAS servers)

Figure 7: Select a configuration file (ccf file). See text for details.
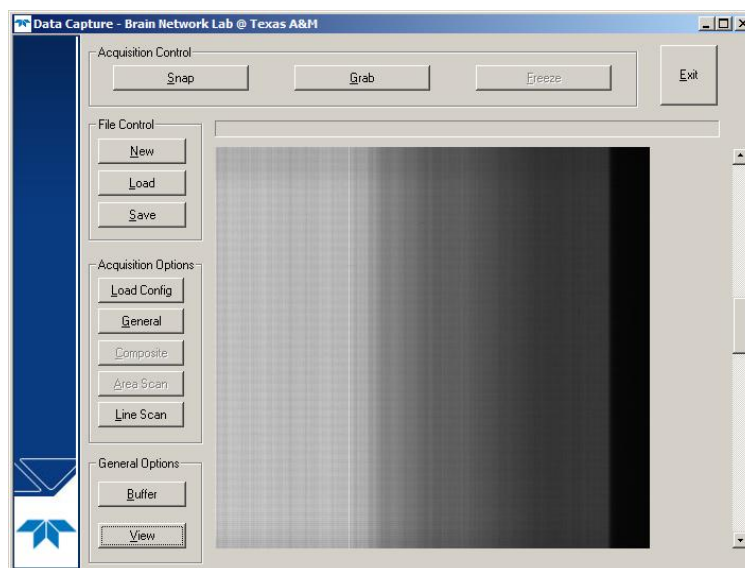

Figure 8: KESM Data Capture GUI

2 U500mmi

1. Make sure stage controller cables are connected

2. Turn on stage controller

3. Check air pressure gauges to stage

4. Locate and start U500mmi application (via start menu or desktop icon)

5. Go to menu and select parameters

6. Navigate to fine kesm.prm

7. Click the reset button at bottom right corner

8. Enter 'pr me un/se' into command window to change program to meter units per second rather than per minute (which is default)

9. Make sure stage is in the center with X and Y axis and the knife and objective assemblies are up

10. Enable the X, Y, and Z axis on the stage controller using the top left buttons (the three axis lights on the stage controller should be on and the stage locked in position afterward)

11. Home the Z, Y, and X axis IN ORDER using the top buttons (the stage will move along each axis gauging its range before resting at position 0 for each axis respectively) NOTE: the X axis will fluctuate a little due to it being moved on air

12. Click the Jog button to enter the movement mode for the controller software

13. In the new window, set the Speed of the respective axis to be: 20 for X, 5 for Y, and 1 for Z

14. Have the stage move to the central position of X=155 and Y=22 by holding down axis' + and - buttons respectively (it will move until either it has traversed the range set from where you started or you release the button) FYI: the space key will allow you to click whatever button you previously selected

15. When preparing for cutting tissue you need the bounding rectangle in the X and Y axis as well as a starting Z point slightly above the tissue

    - Move the stage to two diagonal corners extents of the bounding block and record their X and Y coordinates (FEEDBACK). NOTE: make sure you have the bounds for the entire cutting as re-alignment of images later can be a pain.
    - A quicker approach can be used by disabling a single axis (X or Y only) and repositioning by hand, but be aware this makes ALL BUT THE FEEDBACK DATA INVALID
    - For acquiring the Z position one can position the knife over the stage and move the stage up in increments of 0.1 distance until close (if holding the light above the knife you should see a shadow come into focus below on the tissue), then switching to 0.01 for potential collision. If the to much distance is used in the Z direction the knife can be dislocated in its the housing.
    - Another approach for the Z axis is to disable the X axis and move the Z axis up while manually 'slicing' across the block until a collision occurs (DO NOT USE MUCH FORCE WHEN PUSHING THE KNIFE OVER THE TISSUE)
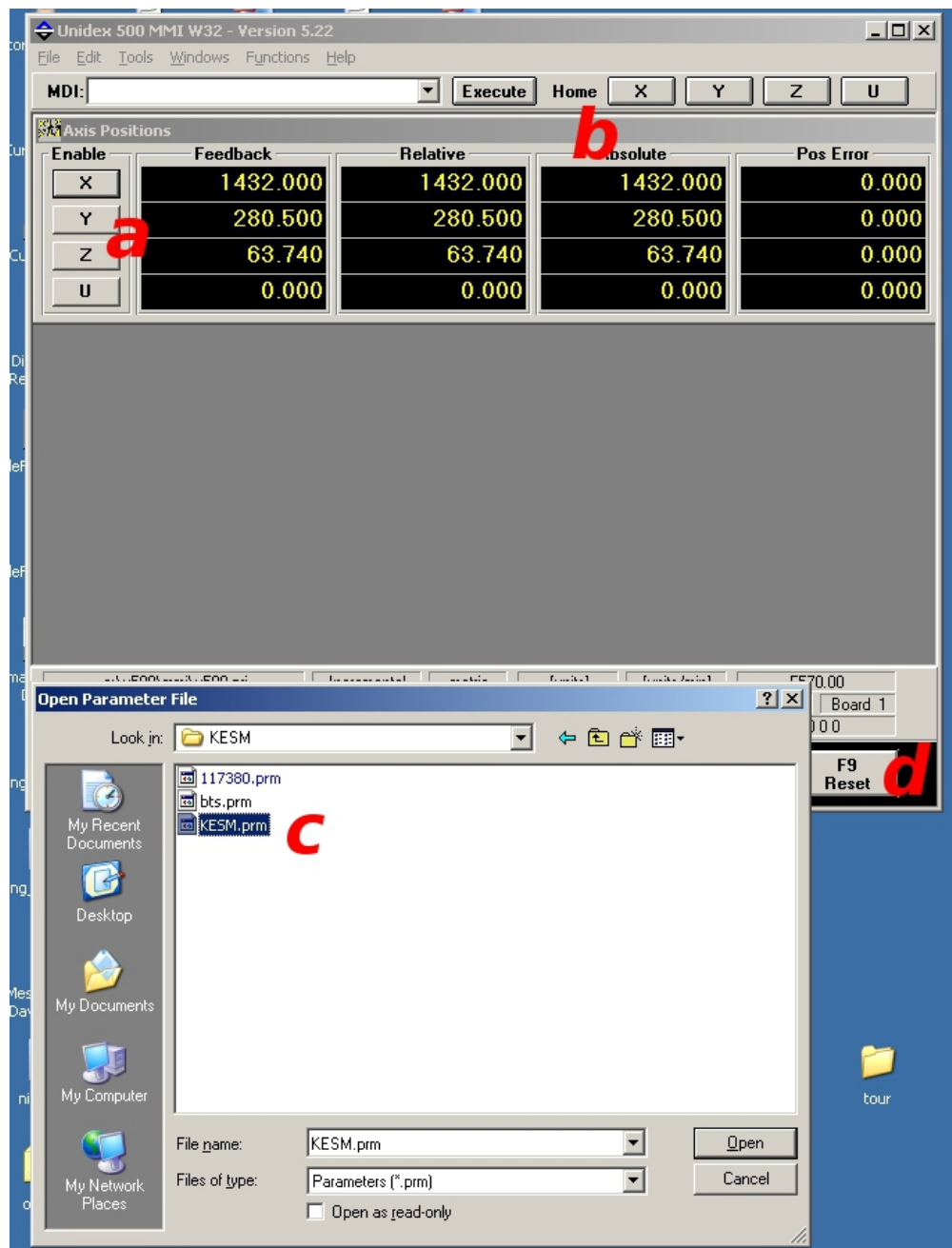
Figure 9: U500mmi: a) axis enabling buttons, b) axis homing buttons, c) parameter loading, d) reset button for stage
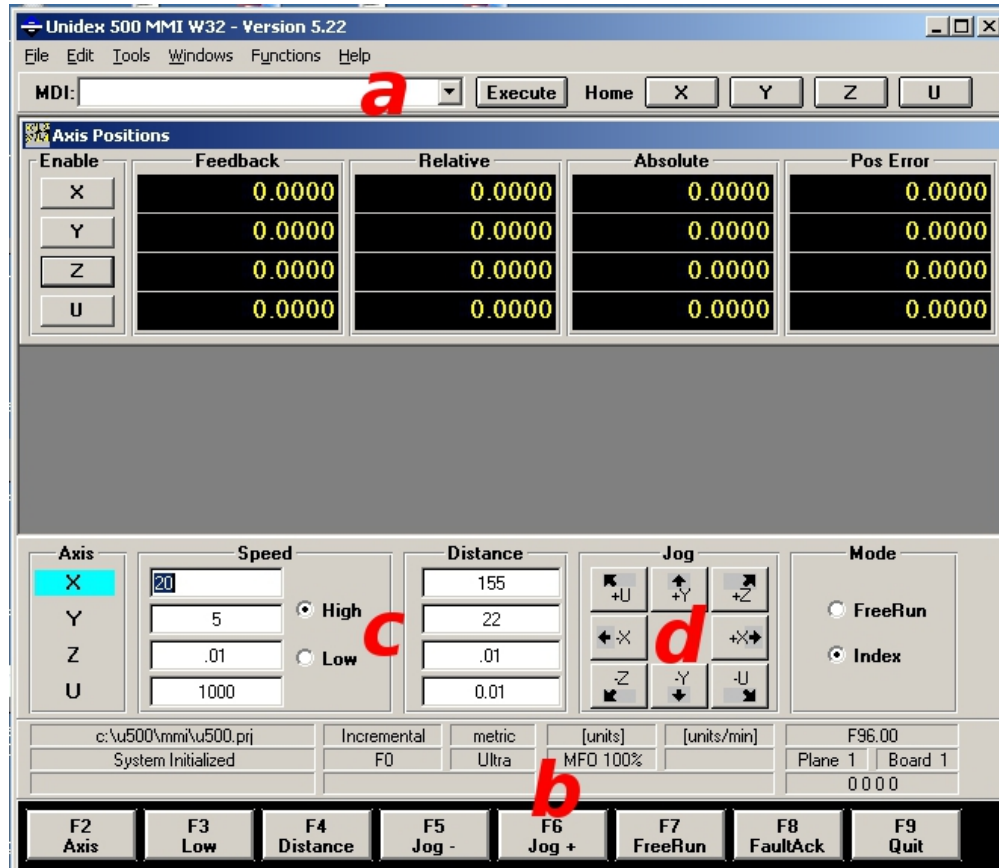
Figure 10: U500mmi: a) input command window, b) jog button, c) speed and distance parameters for jogging, d) movement buttons for jogging
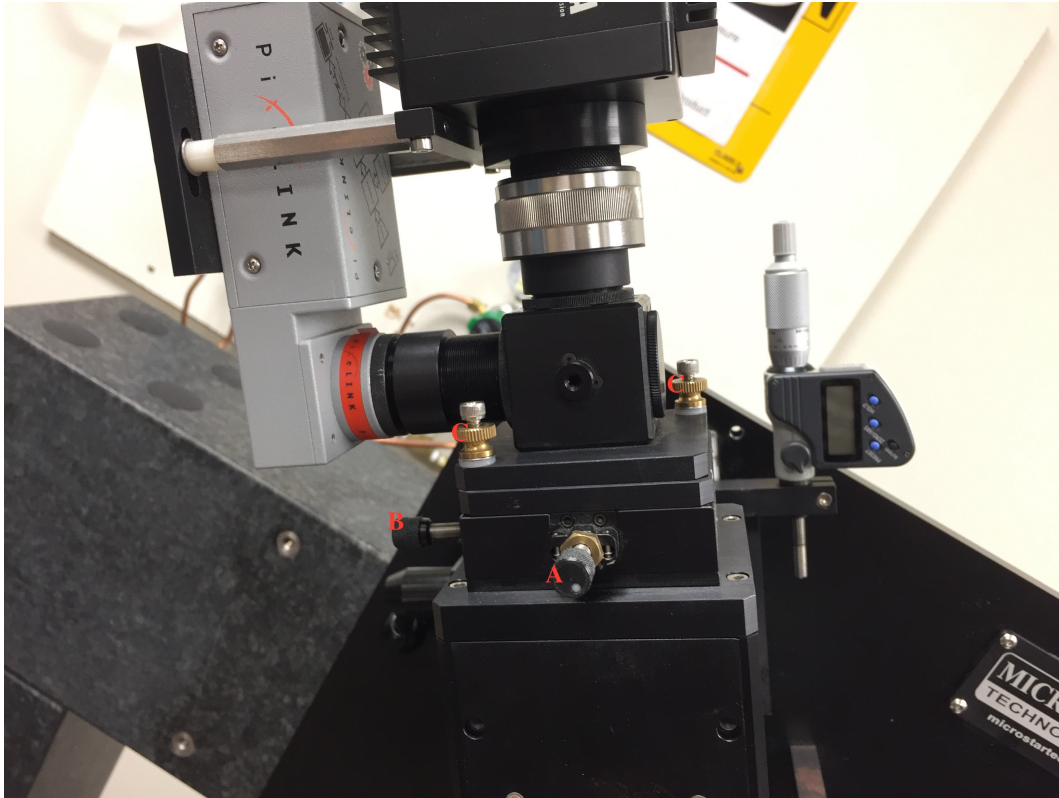
3 Camera focusing

Figure 11: Linescan camera adjustment: A) adjust camera view left to right. B) adjust camera view top to bottom (top is toward the cutting direction). C) Slightly loose these two knobs before adjusting the camera. Remember both axis are with respect to what one can see through the camera GUIs.

- Use both the side camera (PixeLink captureOEM) and the line scan camera (Sapera CamExpert) for camera adjustment.

- For an initial focus, getting the knife tip in focus through the viewer is good (though for cutting this needs to be fine tuned due to the camera and viewer not being in the exact same focal range)

- Moving the camera UP makes the camera look through the objective towards the knife tip from the base of the knife (DIMMER, BETTER FOCUS) (see Fig.11)

- Moving the camera DOWN makes the camera look through the objective towards the knife base from the tip of the knife (BRIGHTER, WORSE FOCUS) (see Fig.11)

- To initially get the camera focused on the tip, make sure the illuminator is set on the highest setting and move the camera to toward the knife tip till it runs off (image will be all black). Then you can move the camera toward the base until you see the illuminated knife (see Fig.11)

4 Knife cleaning

1. Remove the knife from the KESM by unscrewing the nut keeping the mount in place and unplugging the fiberoptic cable from the illuminator

2. Transfer the knife to the specimen microscope

3. Locate a hair tipped brush and the jars of trichoroethylene and methyl ethyl ketone

4. You should use the latex gloves to prevent the compounds from getting on your skin (wash thoroughly if it does get on you)

5. Use the brush and the microscope's view to brush on the trichoroethylene on the front and back of the knife (should break up what is on the knife)

6. Rinse off the brush

7. Use the brush and the microscope's view to brush on the methil ethyl ketone on the front and back of the knife (should appear a lot clearer and uniform)

8. Rinse off the brush

9. Place mount back on KESM and screw it down, plugging in the fiberoptic cable to the illuminator

5 Knife alignment

- Attach the knife to the arm if it is not already done

- Lower the knife arm (don't lock)

- Carefully lower the objective arm to ensure it doesn't hit the knife (if the objective would hit, increase the distance from objective to the knife)

- Use the knob at the top of the knife mount to adjust the position of the knife to where it is about halfway across the objective field (see Fig. 12(a) and Fig. 12(c)).

- Try locking the knife position and seeing if the objective field needs more adjustment

- In the case the knife corner can't be seen (see Fig. 12(d)) or the knife tip is not all in focus upon focusing on one part (see Fig. 12(c) and Fig. 12(d)), adjust the knife yaw and pitch respectively see Fig.13)
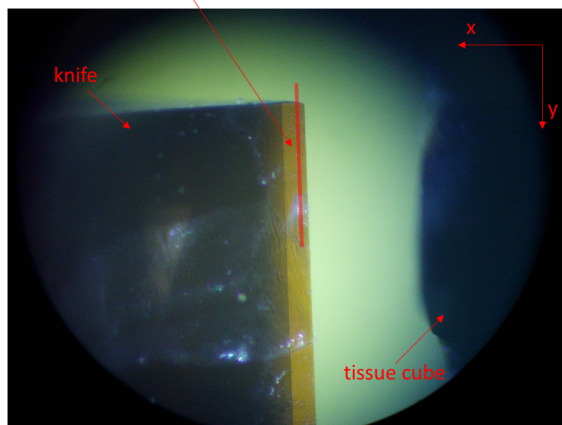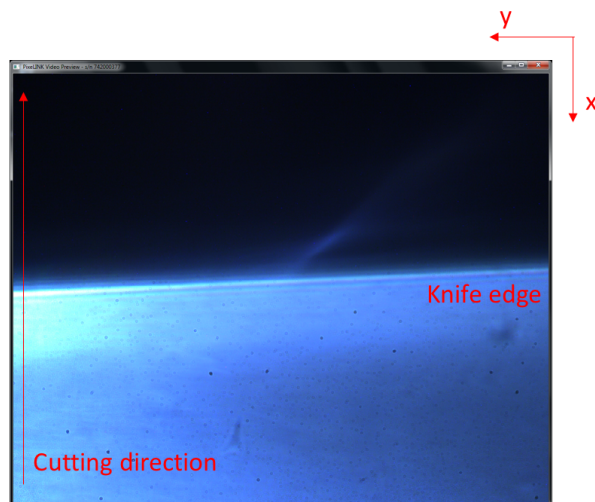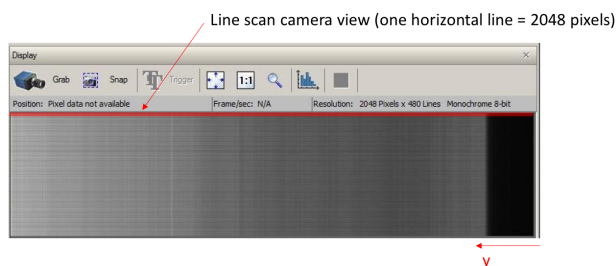
(a) View of the knife through the viewfinder (KESM 1.0)
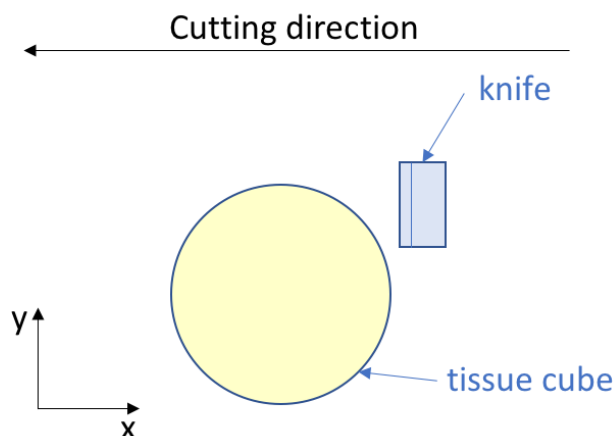
(b) Illustration of the overhead view of the knife

(c) Side camera view (PixeLink captureOEM GUI)

(d) Linescan camera view (CamExpert GUI)

Figure 12: **Different views of the knife for alignment.** (a) View of the knife through the viewfinder (KESM 1.0). Though KESM 2.0 does not have the viewfinder, this image can give an idea of how the knife can be aligned. (b) Illustration of the overhead view of the knife and a tissue cube. Compare this illustration with the other views (c and d) to see how the knife image is transformed in the camera views. (c) The side camera view of the knife in the PixeLink captureOEM GUI. (d) The linescan camera view of the knife in the CamExpert GUI.
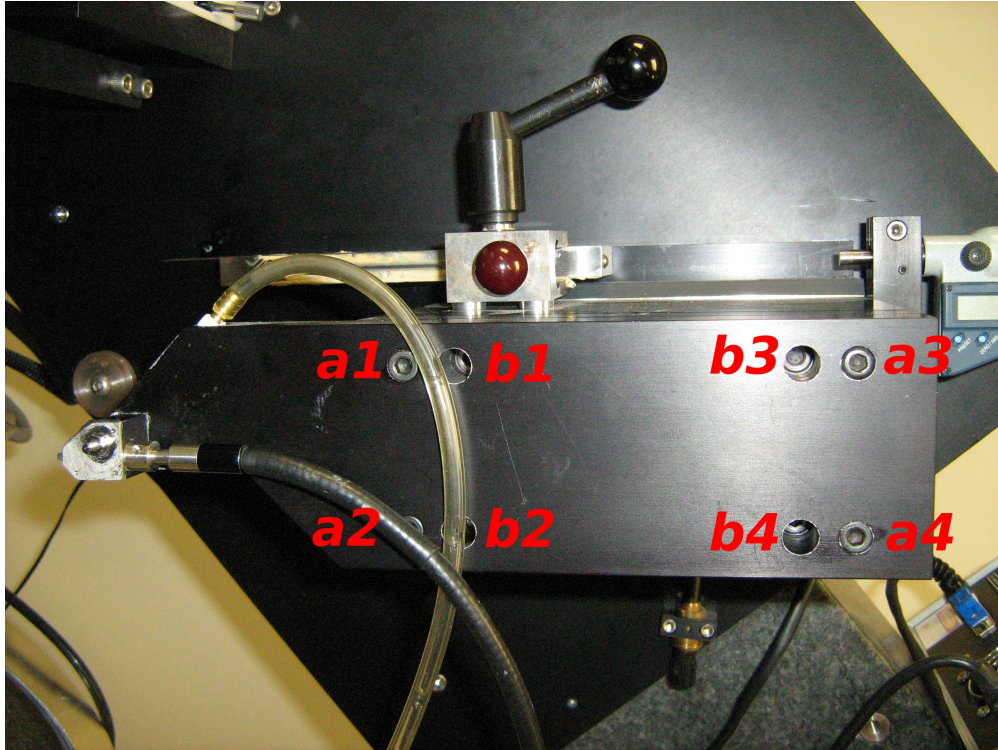
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Figure 13: Knife alignment adjustment: a) adjust the knife roll, b) adjust knife yaw
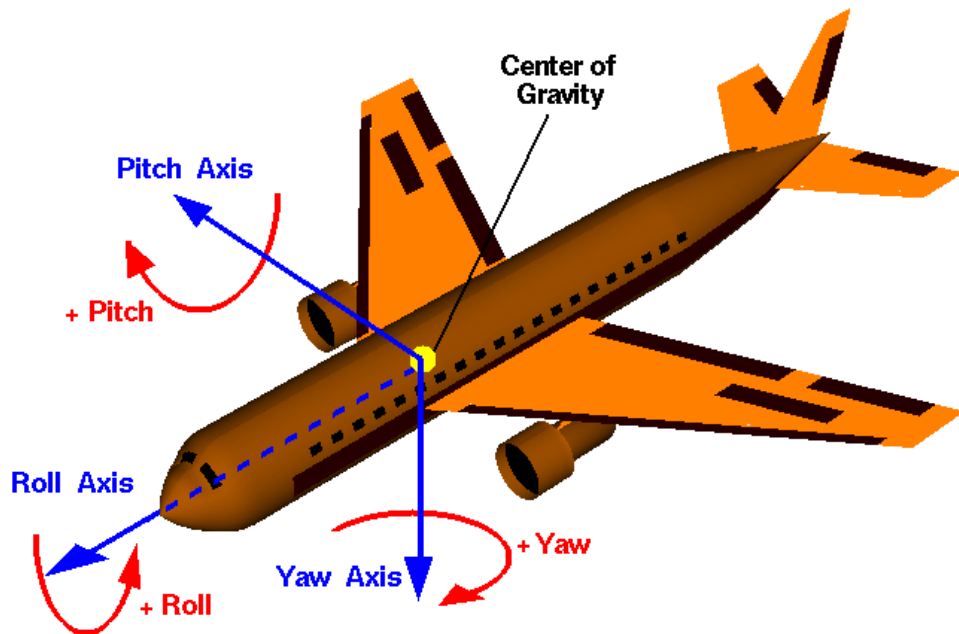

Figure 14: Roll, pitch, and yaw diagram courtesy of wikipedia

6 Water System

- Make sure knife arm is down and tissue entry into knife is covered by water

- Make sure manual pump connection is closed

- Turn on the pump to get the water going toward the pump, then turn off when it stops being effective

- Quickly open the connection and use the manual pump to suction out the air in the pump and pull the water to the pump

- If the air starts moving back towards the stage then close the connection, turn on the pump and try again

- TIP: this works best when you have one person manning the connection and the other manning the manual pump (priming as fast as possible)

7   Common Problems

1. Pump

2. disk space

3. tissue blade clearance

4. focus (using knob)

5. cutting with low light