SCOUT Version 1.0

1. Download and Install Instructions
   - Using the source code: Download the source code from github and run the application through matlab 2021a (scout_v1.mlapp) and standalone versions are available. Make sure you have the Image Processing Toolbox and Statistics and Machine Learning Toolbox installed as well.
   - Using the standalone versions:
     - If you have the MATLAB 2021a already installed, you can just open the standalone app (SCOUT.exe)
     - If not, you will need to install MATLAB 2021a runtime at https://www.mathworks.com/products/compiler/matlab-runtime.html and then run the standalone app (SCOUT.exe)
2. Folder/File Structure: The application requires a specific folder and file structure as depicted below. This includes an information file that contains information for each image (subject name, filename, which side is medial in the image, limb, resolution in X direction, resolution in Y direction (mm/px). This file is saved as the 'StudyName_**StudyInfo.csv**'

   Example: Main study folder = 'EATA', subjects = EATA001, EATA002, study info file = 'EATA_StudyInfo.csv'.

   › EATA

Name	Date modified	Type	Size
EATA001	8/18/2021 11:59 PM	File folder	
EATA002	8/18/2021 11:59 PM	File folder	
EATA_StudyInfo.csv	8/19/2021 12:00 AM	Microsoft Excel Co...	1 KB

   a. The study info file needs to be a csv file structured as below with all other cells. If you get an error upon loading, make sure there is not any information in surrounding cells.

Patient ID	Image Filename	Limb	Medial Side	Resolution X	Resolution Y
EATA001	Cartilage1.dcm	R	R	0.0902	0.0902
EATA001	Cartilage2.dcm	R	R	0.0902	0.0902
EATA002	L6GD6H80	R	R	0.0902	0.0902
EATA002	Cartilage9.dcm	R	R	0.0902	0.0902
EATA002	1.jpg	R	R	0.0902	0.0902

   b. Accepted Image formats: dcm, jpg

   *Check out the example_study provided on GitHub. After downloading the example folder, open up the application and choose the example_study folder to load the project. You can then see how to set up the folder structure and example_study_StudyInfo.csv file.

3. Usage

a. A mat file is created for each image that contains the image setting and processing information, and data so upon loading an image, you will see that a mat file is created at the same file path.
b. **Image Settings Panel:** This panel has the cropping and set corners buttons. Once cropped, this data is saved in the mat file so the cropping will be saved when reloading an image. The set corners button is important for those who want to apply a tissue correction factor. Currently the corners are used to determine the thickness from the top of the image to the near contour. There is an attempted auto-detect corners but it uses pixel intensities to try to identity the corners but given that users will have different image backgrounds, this is unlikely to work for all cases. Be sure to adjust the corners before taking measurements. When you click this button, two points will appear with the X,Y coordinates after you click and drag each point. If you don't plan to use a tissue correction factor, you can ignore these points.
c. **Image Enhancement Panel:** This panel has contrast and filtering options to better detect contours in the image. Note that all grayscale/echo intensity values are taken from a gray scale copy of the image so this is purely to better define the contours to make tracing more feasible.
d. **ROI options Panel:** This panel contains the main drawing of the contours, and options to adjust the contours and intercondylar notch. You first need to choose how many contours you want to draw and ensure this is consistent throughout the study. Choosing to draw 'Two' contours assumes the traditional far quadriceps tendon-cartilage (contour 2) and near cartilage-bone interface (contour 3) contours. Choosing to draw 'Three' contours assumes the near far quadriceps tendon-cartilage (contour 1), along with far quadriceps tendon-cartilage and near cartilage-bone interface (contour 2-3). You will always draw the contours from top to bottom sequentially meaning that you will draw the near quadriceps tendon-cartilage, then far quadriceps tendon-cartilage, then near cartilage-bone. If you only draw 2 contours, you will still follow this approach. To draw a contour, left click to draw each vertex of the contour and then when you finish drawing a contour, right click to move to the next contour. When you finish all contours, you are able to edit the contours by deleting vertices, adding vertices (double click or right click), or repositioning vertices. Once you have ensured correct positioning, click the the 'Accept Contours' button that will appear after you finish drawing.

   Once you click accept, the contours will be interpolated (spline) and then they will appear with the point of the intercondylar notch that is estimated by the deepest part of contour 3 (bone-cartilage). The intercondylar notch is used to center the regions of interest and ensure the medial and lateral regions are equal in length. If you want to adjust the contours or the intercondylar notch, click 'Adjust Contours' or 'Adjust Notch' and move the ROI to the corrected position. Note that 'Adjust Contours' will allow repositioning from the most recent draw made.
e. **Measurement Options Panel:** This panel is where you choose the size of the intercondylar notch, number of sub regions, how to ensure consistency in lengths between the medial and lateral sides, and correction factors for speed of sound and refraction.

For the intercondylar percentage, you can choose a number between 0-50. A value of 0 means there is not intercondylar region, so you are analyzing only medial and lateral regions.

For the number of sub regions, you can choose a number between 1-10. Assuming the intercondylar region is not 0, a value of 1 means you split the cartilage into whole regions (medial, lateral, and intercondylar) only. Whereas a value of 3 means you split each whole region into 3 sub regions so that you would have 3 sub regions of the whole medial, 3 sub regions of the whole lateral, and 3 sub regions of the whole intercondylar for a total of 9 sub regions and 3 whole regions.

For the M/L Length, we want to ensure the medial and lateral lengths are equal in length. There are 2 options for how to do this. 1) Minimum Distance: the minimum distance of either the medial or lateral side is used. This is done from what is drawn so be sure to draw the contour appropriately. Some researchers set the medial and lateral lengths to be 10mm in length from the intercondylar notch to so that option is present as well.

The speed of sound correction factor is used in the refraction correction for when the ultrasound beam meets the cartilage interface (either the near or far quadriceps tendon-cartilage depending on if you choose to draw 2 or 3 contours). A suggested factor of 1.10 has been used to correct for the speed of sound in the 'tissue' vs. speed of sound in cartilage [(1696 (m/s) / 1540 (m/s)]. However, it is important to note that these speed of sounds for cartilage and biological tissue are only estimates and a wide range has been reported in the literature. Also, this correction operates under the assumption that the speed of sound in different tissue types is uniform when it is known to be anisotropic and impacted by multiple biological and ultrasonic factors.

Refraction input is determined by identifying the number of slope changes to best fit the contour to then correct for overestimation of thickness caused by the ultrasound beam meeting the cartilage interface at oblique angles in each one of the sloped ROIs.

f. **Display Results Panel:** This panel is self-explanatory but be sure to check these prior to saving the data. Make sure your contours, intercondylar region, ROI's, and image corners are correct before moving to the next image or onto saving and processing. You can change the colors of these by clicking the color icon. Don't forget to check image corners if you plan to use a tissue correction factor.

g. **Process, Save, & Compile Panel:** This panel has options for processing and compiling the data.

   The Process and Save drop down is to provide an option to process a **Single File** or **All Files** at once. Choosing a **Single File** will process and save the data for the current image.

Processing **All Files** assumes you have drawn, measured, and checked the ROI's and image corners on all images in the study or multiple images in the study. This function will go through every image with a corresponding mat file in the study folder as long as it has the same number of contours drawn. It will load each image that fits the criteria (drawn contours), take the measurements with the user defined options in the app (intercondylar percentage, number of sub regions, M/L length option, and correction factors) and process all of the files at once rather than processing each image at the time of drawing and measuring.

There isn't a right way to do this - this is more of a convenience option and helps to ensure consistency in the user-defined options across all images. It can also be a way of having a second or third person go through and check the contours to ensure they are drawn correctly and consistently prior to processing all of the data at once.

The compile button is used to compile all data from a study on 1) an image-by-image basis AND 2) an average by subject and limb. Many researchers take multiple images of the same limb and then average the data together for that subject and limb so the 'Average by Limb' option does this for you.

In summary, the process and save button will save all data to the image mat file. The compile button will read each matfile and compile the data into 2 master files..

List of all outcome measures. These change depending on if you draw 2 or 3 contours, and if you have an intercondylar ROI. If you choose to have sub-regions, all outcomes are taken from the whole ROI and each sub-ROI.

Cartilage Outcomes

Minimum, Average, and Maximum Thickness: This uses the distance2curve function that finds the linear distance between points on each contour. If you draw 2 contours, thickness is taken between far quadriceps tendon-cartilage and near cartilage-bone interfaces. If you draw 3 contours, two thickness measures are given between A) the near quadriceps tendon-cartilage and near cartilage-bone interfaces and B) the far quadriceps tendon-cartilage and near cartilage-bone interfaces. Units = mm

Minimum, Average, and Maximum Echo Intensity: gray scale value from 0-255. Echo intensity is only taken from between the far quadriceps tendon-cartilage and near cartilage-bone interface as only thickness of the near to far quadriceps tendon-cartilage interface is meaningful, to date. Units = unitless

Minimum, Average, and Maximum Normalized Echo Intensity: all values are normalized with linear scaling to use the full dynamic range by first subtracting the minimum grayscale value from all pixel intensity values, and then dividing by the difference between the minimum and maximum pixel intensity values (if this difference is greater than zero). The resulting normalized intensities will range from 0 to 1, with 0 (black) representing the lowest value and 1 (white) representing the highest. This enables better comparison of echo intensities across images.

Tissue (image to top contour) Outcomes

Tissue distance (above each ROI): The amount of tissue above each ROI can impact the echo intensity. Researchers have attempted to account for this with different correction factors but no consistent method has outperformed others. Therefore, we save the thickness from the top of the image to the top of the near contour. Units = mm

Structural Outcomes

Trochlear Depth and Sulcus Angle: The peaks of each condyle and the apex of the femur are found using the near cartilage-bone contour. From these peaks we can gather the depth and sulcus angle of the femur.

***All measures are influenced by the quality of the image (rotation, transducer orientation) and user defined contours. Optimal data collection set up, ultrasound parameters, personnel image processing trainings should be given proper attention.**