

LinkAn

Linkage value analysis application for fracture callus

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Toolbox Requirements

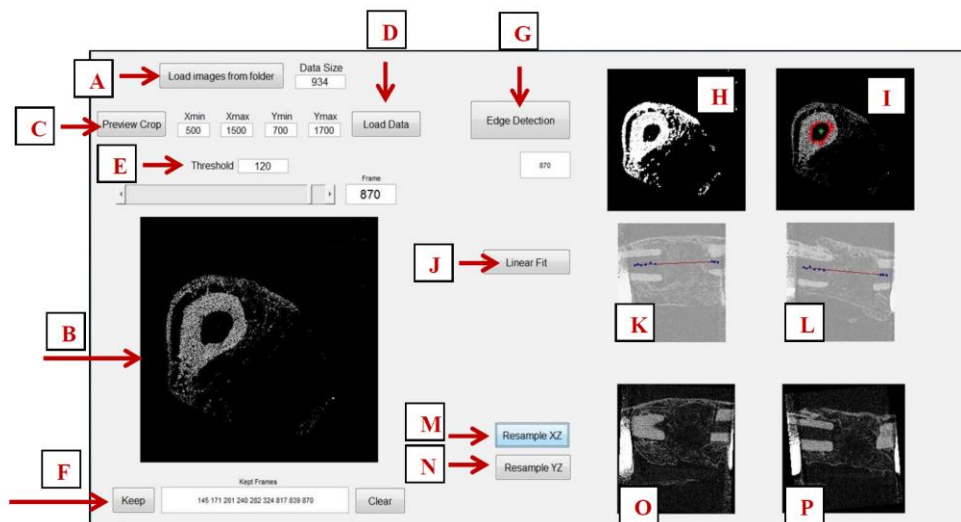
- Image Processing Toolbox Version 11.2 or higher
- Symbolic Math Toolbox Version 8.6 or higher

Rotate Bone

This tab is used to rotate image files so they are parallel with the z-axis.

Preparing your data:

Place greyscale image files into a separate folder. Make sure There are only image files in this folder.



1. (A) "Load Images from Folder" button. The user was prompted to select a folder of images to upload.
2. (B) Once images were loaded, the first transverse section in the image set was displayed here.
3. (C) The entire image set can be cropped to eliminate excess rows and columns. The "Preview Crop" button allowed the user to see how the cropped data set will look before being cropped.
4. (D) The "Load Data" button cropped the image set and the newly cropped data was displayed in window B.
5. (E) The minimum threshold was set here. All voxels with a value below this value were set to 0 and appeared black. The threshold was set such that a clear outer periphery of the cortical bone could be defined.
6. (F) Desired images were selected by using the "Keep" button. The centroid of kept images was located and stored.
7. (G) The "Edge Detection" button initiated MatLab's edge detection function.
8. (H) All defined edges from one image will be outlined in white and displayed here.

9. The femoral canal edge (red) and the centroid associated with this edge (green) were displayed here.
10. (J) Once the centroid from all selected images had been identified, the “Linear Fit” button was used to display the best-fit line through all the centroids in the x-z (K) and y-z (L) planes. Centroids were displayed in blue and the best-fit line was displayed in red.
11. (M) The “Resample XZ” button rotated the image set in the x-z plane such that the linear fit from (K) was

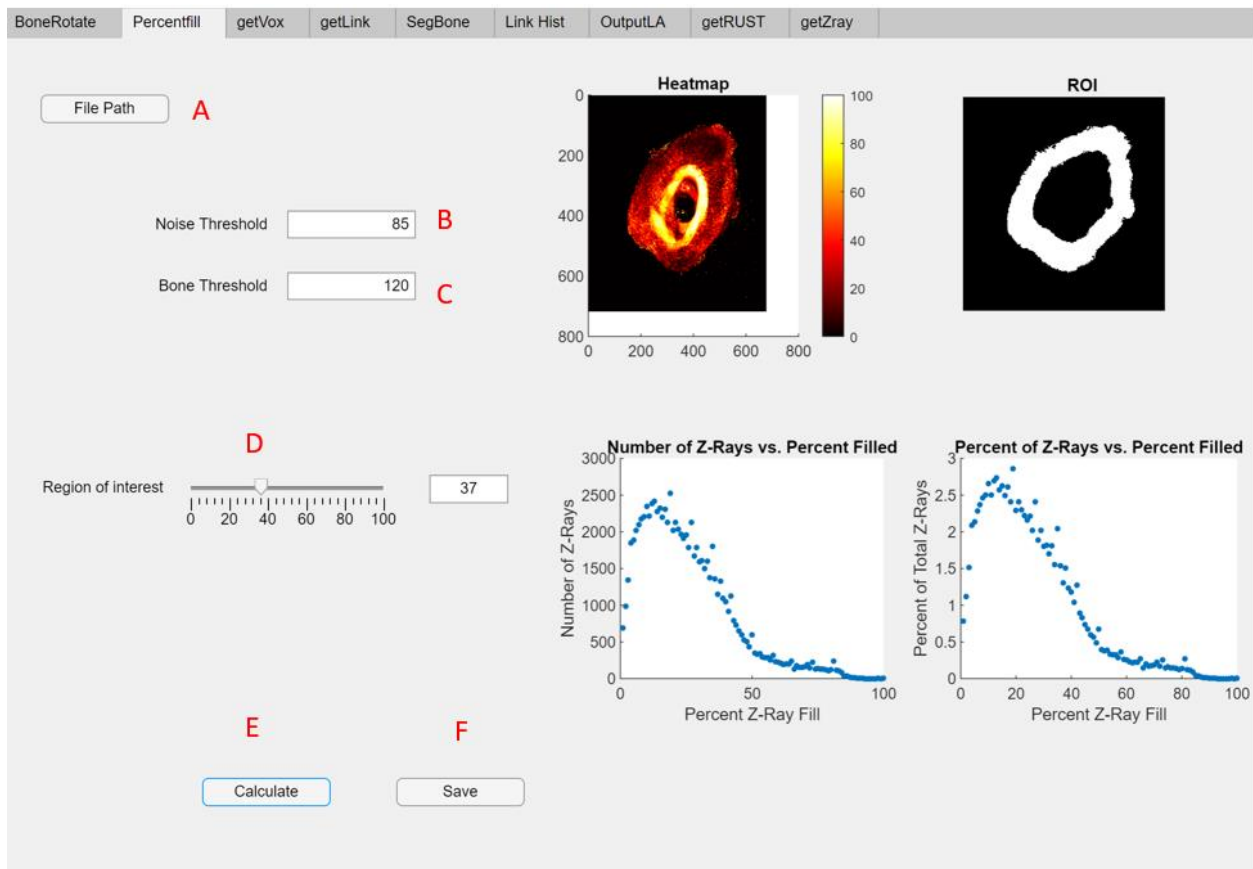
parallel to the ground. (O) Rotated images in the x-z plane were displayed here in real time.

12. (N) The “Resample YZ” button rotated the image set in the y-z plane such that the linear fit from (L) was parallel to the ground. (P) Rotated images in the y-z plane were displayed here in real time.
13. Press Create slice to output new image files.

PercentFill

This tab is used to calculate percent fill of zrays in a fracture callus

Preparing your data: Place greyscale images into a folder. There should only be images in this folder.



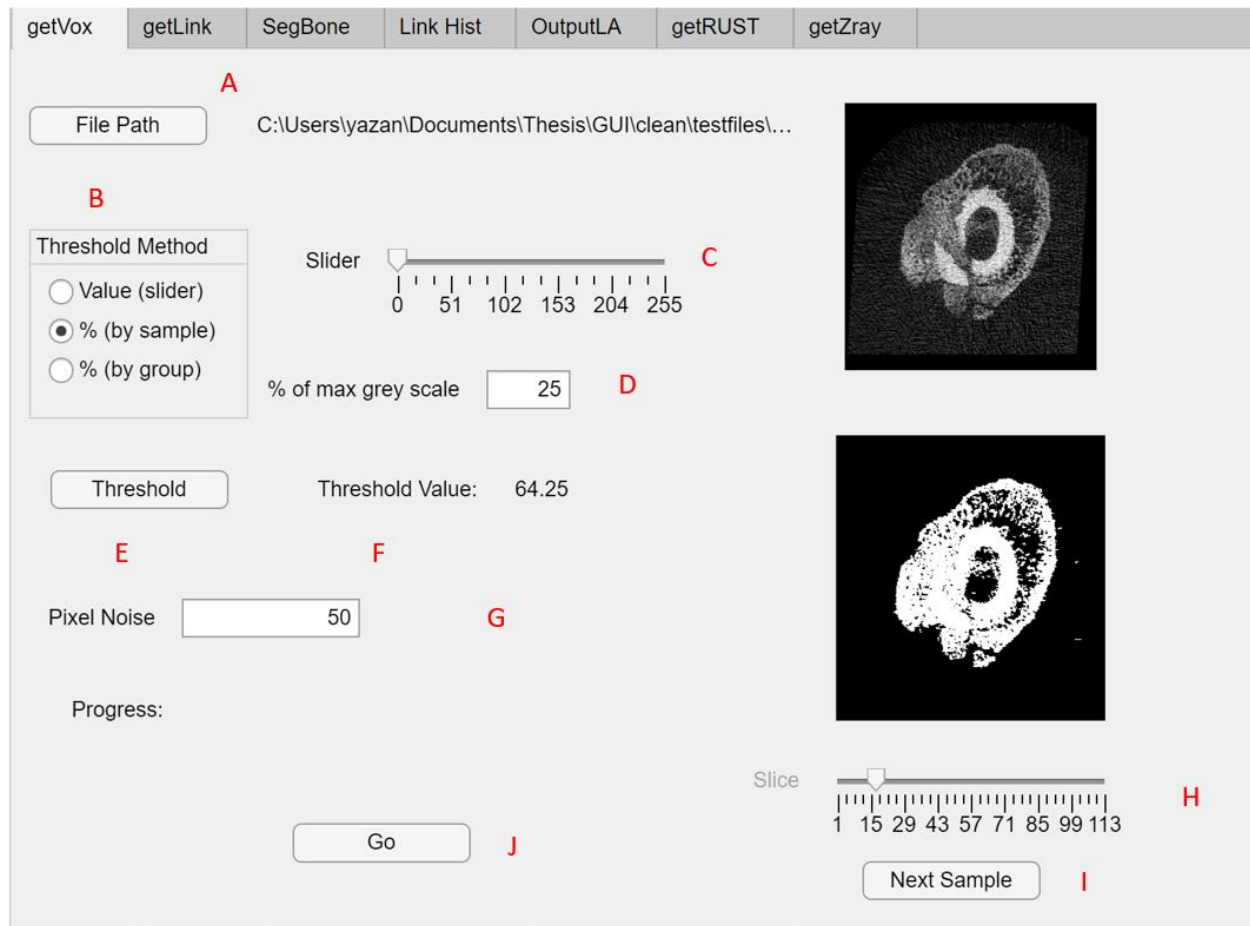
1. Press file path to select folder with images.
2. Insert noise threshold. This grayscale value represents tissue density.
3. Insert bone threshold. This greyscale value represents bone.
4. Select a region of interest. This is a percentage of the total callus perimeter.
5. Press Calculate and check graphs and ROI
6. When satisfied with ROI and heatmap, press save to save files.

getVox

This tab is used to threshold greyscale μ CT images and save them in a vox file.

Preparing your data:

Images should be transverse slices oriented with the diaphysis parallel with the sagittal and coronal planes. Images should be cropped with no boarder. Images for a given sample should be saved in a separate folder named with a unique sample ID. Each sample folder should contain ONLY image files. All sample folders should then be placed in a folder. Again, the only files in these folders should be images.



Steps:

1. Click file path (A)
2. Select folder containing sample folders
 - a. Greyscale image will load onto top window
3. Select threshold method (B)
 - a. Value: Threshold limit set by value on slider (C)
 - b. %(by sample): Threshold limit set as user inputted % of maximum greyscale value (D) for each sample

- c. %(by sample): Threshold limit set as user inputted % of maximum greyscale value (D) for all samples
4. Insert pixel noise threshold (G)
 - a. This will remove object in image equal to less than noise threshold
5. Click threshold (E)
 - a. Calculated threshold value will display (F)
6. Visualize threshold images in lower display pane.
 - a. You can scroll through sample slices with slider (H)
 - b. To view next sample, click next sample (I)
7. When satisfied with thresholding, click Go (J)
8. Samples will be saved into _Vox.mat files

getLink

This tab will automate calculating linkage value matrices for _Vox.mat files.

Preparing your data:

Take all _Vox.mat files from the previous section and place them into a new folder. The only file type in this folder should be _Vox.mat.

Steps:

1. Click File path button.
2. Select folder with _Vox.mat files.
3. Click Go
4. Program will save _Link.mat files

SegBone

This tab will automate segmentation of callus and cortical bone.

Preparing your data:

Take all _Link.mat files from the previous section and place them into a new folder. The only file type in this folder should be _Link.mat.

Steps:

1. Click File path button.
2. Select folder with _Link.mat files.
3. Click Go
4. Program will save _BonePart.mat files

LinkHist

This tab will automate calculating linkage value histograms for the fracture callus.

Preparing your data:

Take all _BonePart.mat files from the previous section and place them into a new folder. The only file type in this folder should be _BonePart.mat.

Steps:

1. Click File path button.
2. Select folder with _BonePart.mat files.
3. Click Go
4. Program will save _ConnhHist.mat files

OutputLA

This tab will display linkage area parameters calculated in the previous tab.

Preparing your data:

Take all _ConnHist.mat files from the previous section and place them into a new folder. The only file type in this folder should be _ConnHist.mat.

getVox

getLink

SegBone

Link Hist

OutputLA

getRUST

getZray

A

File Path

Progress:

B

cut off

1

6

11

16

21

27

C

Direction

☒ Forward
 ☐ Backward

D

Go

Steps:

1. Click File path button (A).
2. Select folder with _ConnHist.mat files.
3. Select threshold level (B).
4. Select direction of linkage area thresholding
 - a. Forward-This will calculated linkage area from 1 to threshold level set in step 3
 - b. Backward-This will calculated linkage area from 27 to threshold level set in step 3
5. Click Go (D)
6. Linkage area parameters will display in chart panel

getRust

This tab will automate calculating rust scores for fracture callus samples.

Preparing your data:

Take all _BonePart.mat files from the previous section and place them into a new folder. The only file type in this folder should be _BonePart.mat.

Steps:

1. Click File path button.
2. Select folder with _BonePart.mat files.
3. Click Go
4. Program will display RUST scores in chart panel

getZray

This tab will automate calculating zray distribution samples and output zray file and heatmap.

Preparing your data:

Take all _BonePart.mat files from the previous section and place them into a new folder. The only file type in this should folder to should be _BonePart.mat.

getVox getLink SegBone Link Hist OutputLA getRUST getZray

A

File Path

B

Z-Ray Method

☒ Longest Contiguous

☐ Total

C

Region of Interest

☒ Total

☐ Callus

☐ Cortex

Name	10	20	30	40	50	60	70	80

D

Go

Steps:

1. Click File path button (A).
2. Select folder with _BonePart.mat files.
3. Select Z-Ray method (B)
 - a. Longest contiguous: this will calculate longest contiguous zray length for a given X,Y coordinate
 - b. Total: this will calculate total number of positive voxels for a given X,Y coordinate
4. Select ROI (C)
 - a. Total: Total callus fracture
 - b. Callus: Callus area only
 - c. Cortex: Cortical bone only
5. Select Go (D)
6. Program will calculate Zrays for each sample.
 - a. A heatmap figure will be saved
 - b. A _Zray.mat file will also be saved
7. Number of zrays in each decile for each sample will be displayed in chart panel.

File Types:

_vox.mat

File Type	_vox.mat
Structure name	bone
Variable	Description
bone(1).name	Returns sample name
bone(n).vox(x,y)	Returns 2-D array representing CT slice. N denotes slice number in z direction. X and Y represent x and y coordinate of sample image

_link.mat

File Type	_Link.mat
Structure name	rotatedbone
Variable	Description
rotatedbone(1).name	Returns sample name
rotatedbone(n).vox(x,y)	Returns 2-D array representing linkage value. N denotes slice number in z direction. X and Y represent x and y coordinate of sample image
File Type	_ConnHist.mat
Structure name	Bone
Variable	Description
Bone(1).name	Returns sample name
Bone(n).ROI	Returns Description of region of interest for TV and histogram readings. n ranges from 1-6 and corresponds to: "Callus", "Inner", "Total", "Effective Callus", "Effective Inner", "Effective Total" respectively
Bone(n).TV	Returns tissue volume for all slices in sample. N denotes ROI
Bone(n).TVcanal	Returns canal volume for all slices in sample. N denotes ROI
Bone(n).hist1	Returns a histogram of linkage values for a given slice n for the callus object. n begins at a value of 2 and continues to total number of slices-1

Bone(n).peak1	Returns most frequency linkage value for a given slice n for the callus object. n begins at a value of 2 and continues to total number of slices-1
Bone(n).hist2	Returns a histogram of linkage values for a given slice n for the cortical object. n begins at a value of 2 and continues to total number of slices-1
Bone(n).peak2	Returns most frequency linkage value for a given slice n for the cortical object. n begins at a value of 2 and continues to total number of slices-1
Bone(n).hist3	Returns a histogram of linkage values for a given slice n for the total object. n begins at a value of 2 and continues to total number of slices-1
Bone(n).peak3	Returns most frequency linkage value for a given slice n for the total object. n begins at a value of 2 and continues to total number of slices-1
Bone(n).hist4	Returns a histogram of linkage values for a given slice n for the effective callus object. n begins at a value of 2 and continues to total number of slices-1
Bone(n).peak4	Returns most frequency linkage value for a given slice n for the effective callus object. n begins at a value of 2 and continues to total number of slices-1
Bone(n).hist5	Returns a histogram of linkage values for a given slice n for the effective cortical object. n begins at a value of 2 and continues to total number of slices-1
Bone(n).peak5	Returns most frequency linkage value for a given slice n for the effective cortical object. n begins at a value of 2 and continues to total number of slices-1
Bone(n).hist6	Returns a histogram of linkage values for a given slice n for the effective total object. n begins at a value of 2 and continues to total number of slices-1
Bone(n).peak6	Returns most frequency linkage value for a given slice n for the effective total object. n begins at a value of 2 and continues to total number of slices-1
Bone(n).TVmax	Returns slice with greatest tissue area. N denotes ROI
Bone(n).TVmin	Returns slice with least tissue area. N denotes ROI
Bone(n).TVave	Returns average tissue area. N denotes ROI
Bone(n).histmax(1,X)	Returns 1X27 array corresponding to largest frequency of linkage values from X to 27 in a given slice. N denotes ROI
Bone(n).histmin(1,X)	Returns 1X27 array corresponding to smallest frequency of linkage values from X to 27 in a given slice. N denotes ROI

Bone(n).avehist(1,X)	Returns 1X27 array corresponding to average frequency of linkage values from X to 27 in a given slice. N denotes ROI
Bone(n).histmax2(1,X)	Returns 1X27 array corresponding to largest frequency of linkage values from 1 to X in a given slice. N denotes ROI
Bone(n).histmin2(1,X)	Returns 1X27 array corresponding to smallest frequency of linkage values from 1 to x in a given slice. N denotes ROI
Bone(n).avehist2(1,X)	Returns 1X27 array corresponding to average frequency of linkage values from 1 to X in a given slice. N denotes ROI
Bone(1).TVTotal	Returns TV of total object
Bone(1).TVCortical	Returns TV of coritcal object
Bone(1).TVCallus	Returns TV of callus object
Bone(1).BVTot	Returns BV of total object
Bone(1).BVCortical	Returns BV of coritcal object
Bone(1).BVCallus	Returns BV of callus object
Bone(1).BVTVTot	Returns BV/TV of total object
Bone(1).BVTVCort	Returns BV/TV of coritcal object
Bone(1).BVTVcal	Returns BV/TV of callus object

BonePart

File Type	_BonePart.mat
Structure name	BonePart
Variable	Description
BonePart.name	Returns sample name
BonePart.BreakType	Returns sample fracture type
BonePart.TV	Returns sample tissue volume
BonePart.Centroid	Returns centroid of 2-D compressed view of callus
Bonepart.Callus.obj	Returns 3-D matrix for callus object
BonePart.Callus.objconn	Returns 3-D linkage value matrix for callus object
BonePart.Callus.stat	Returns values from regionprops3('Volume','Centroid','Image', 'VoxelList',Orientation') for callus object.
Bonepart.Inner.obj	Returns 3-D matrix for cortical object
BonePart.Inner.objconn	Returns 3-D linkage value matrix for cortical object
BonePart.Inner.stat	Returns values from regionprops3('Volume','Centroid','Image', 'VoxelList',Orientation') for cortical object.
Bonepart.Total.obj	Returns 3-D matrix for total object
BonePart.Total.objconn	Returns 3-D linkage value matrix for total object
BonePart.Total.stat	Returns values from regionprops3('Volume','Centroid','Image', 'VoxelList',Orientation') for total object.
Bonepart.effCallus.obj	Returns 3-D matrix for effective callus object

BonePart.effCallus.objconn	Returns 3-D linkage value matrix for effective callus object
BonePart.effCallus.stat	Returns values from regionprops3('Volume','Centroid','Image', VoxelList',Orientation') for effective callus object.
Bonepart.effInner.obj	Returns 3-D matrix for effective cortical object
BonePart.effInner.objconn	Returns 3-D linkage value matrix for effective cortical object
BonePart.effInner.stat	Returns values from regionprops3('Volume','Centroid','Image', VoxelList',Orientation') for effective cortical object.
Bonepart.effTotal.obj	Returns 3-D matrix for effective total object
BonePart.effTotal.objconn	Returns 3-D linkage value matrix for effective total object
BonePart.effTotal.stat	Returns values from regionprops3('Volume','Centroid','Image', VoxelList',Orientation') for effective total object.
BonePart.zrayact	Returns 2D array of zray lengths
BonePart.zrayest	Returns 2D array of estimated largest zray location (used for normalization)
BonePart.zraynorm	Returns 2d array of normalized zray for sample
BonePart.zraynorm2	Returns 2d array of normalized zray for peripheral callus only
BonePart.maxconndist	Returns distance from centroid to edge of cortex from centroid
BonePart.xmaxconn	Returns X coordinates to maxconndist
BonePart.ymaxconn	Returns y coordinates to maxconndist
maxconndistcal	Returns distance from centroid to edge of callus from centroid
BonePart.xmaxconncal	Returns X coordinates to maxconndistcal
BonePart.ymaxconncal	Returns y coordinates to maxconndistcal

_Zray.mat

File Type	_.Zraymat
Structure name	bone
Variable	Description
bone.name	Returns name of sample
bone.zray	Returns 2D array with zray lengths
bone.zhist	Returns a histogram of zray frequencies for each decile percentage of callus length