FINAL SCRIPT: APPROVED FOR FILMING

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Title: Longitudinal Micro-Computed Tomography Image Analysis for User-Defined Region of Interest in Critical-Sized Bone Defects

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Author Questionnaire

1. 1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

☐ Correct

2. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

3. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, all done**

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here: **MM/DD/YYYY**
Authors, please provide a tentative date by when you cans hoot the shoot interviews

When you are ready to submit your video files, please contact our Content Manager, Utkarsh Khare.

Current Protocol Length
Number of Steps: 25
Number of Shots: 55 (54 SC)

Introduction

1.1. **Bei Liu:** We develop nanoparticle scaffolds to enhance bone regeneration in critical-sized defects, aiming to improve healing rates compared to traditional scaffolds.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.1.2*

What research gap are you addressing with your protocol?

1.2. **Anthony J. Yosick:** Current methods often track bone volume changes across entire bones, lacking precision in consistently identifying localized regions of interest in longitudinal models.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.2.1*

What advantage does your protocol offer compared to other techniques?

1.3. **Anthony J. Yosick:** Our protocol enables consistent, localized region-of-interest tracking in solid models, improving precision in longitudinal analysis and compared to full-bone volume assessments.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.2.1*

How will your findings advance research in your field?

1.4. **Bei Liu:** These findings will allow us to more accurately quantify bone regeneration over time and more effectively communicate the potential translational impact of our work.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 6.2.1*

Ethics Title Card

This research has been approved by the University of Rochester's Committee on Animal Resources

Protocol

2. Model Alignment for Data Processing

Demonstrator: Victor Z. Zhang

2.1. To begin, open the extracted radius bone from the comparison data set and right-click on it **[1]**. Then, search for **Image Registration Wizard**, and select it **[2]**. In the **Properties** section, set **Data** to the comparison data set for the extracted radius bone **[3]** and **Reference** to the initial time point data set for the extracted radius bone **[4]**.

2.1.2 WIDE: Talent taking a seat at the computer table.

2.1.2 SCREEN: 67904_Screenshot_01.mp4 . 00:10-00:18

2.1.3 SCREEN: 67904_Screenshot_01.mp4 **.** 00:18-00:21

2.1.4 SCREEN: 67904_Screenshot_01.mp4 **.** 00:21-00:30

2.2 In the **Image Registration Wizard Actions** section, click **Skip** for **Step 1 of 4 [1]**. For **Steps 2 and 3 of 4**, use the interact cursor to adjust the TabBox to the common region between the data sets, and click **Apply** under **Action** after each step **[2]**. In **Step 4 of 4**, set **Metric** to **Correlation**, **Transformation** to **Rigid**, **Pre-Alignment** to **Align Principal Axes**, and click **Apply** under **Action** **[3]**.

2.2.1 SCREEN: 67904_Screenshot_01.mp4 . 00:30-00:37

2.2.2 SCREEN: 67904_Screenshot_01.mp4 . 00:40-00:48 and 01:04-01:10

2.2.3 SCREEN: 67904_Screenshot_01.mp4 .01:47-01:54

2.3 After aligning the data sets, right-click on the comparison week data set for the extracted radius bone, search for **Resample Transformed Image**, and select it **[1]**. In the **Properties** section, set **Data** to the comparison week data set for the extracted radius bone, **Interpolation** to **Nearest Neighbor**, **Mode** to **Extended**, **Preserve** to **Voxel Size**, and **Padding Value** to **0**, then click **Apply** **[2]**. A new transformed data set will be generated **[3]**.

2.3.1 SCREEN: 67904_Screenshot_01.mp4 . 03:00-03:11

2.3.2 SCREEN: 67904_Screenshot_01.mp4 . 03:11-03:26

2.3.3 SCREEN: 67904_Screenshot_01.mp4 . 03:26-03:30

3. ROI Selection and Cropping

3.1 Click to turn on the **Ortho Slice** for the initial time point and set **Data** to the initial time point data set for the extracted radius **[1]**. Set **Orientation** so the plane yields a transverse cut through the radius bone **[2]**.

3.1.1 SCREEN: 67904_Screenshot_02.mp4 . 00:18-00:26

3.1.2 SCREEN: 67904_Screenshot_02.mp4 . 00:26-00:35

3.2 Using the **Slice Number** slider in the **Properties** section, adjust the slice number to identify the proximal and distal slices surrounding the critical-sized defect **[1]**. Determine and document the slice number where the fracture meets the diaphysis of the radius bone at both ends **[2]**.

3.2.1 SCREEN: 67904_Screenshot_02.mp4 . 00:45-00:55

3.2.2 SCREEN: 67904_Screenshot_02.mp4 . 01:32-01:42

3.3 Turn on the **Ortho Slice** for the comparison week and set **Data** to the initial time point data set for the extracted radius **[1]**. Then, adjust **Orientation** so the plane yields a transverse cut through the radius bone **[2]**.

3.3.1 SCREEN: 67904_Screenshot_03.mp4 . 00:03-00:16

3.3.2 SCREEN: 67904_Screenshot_03.mp4 . 00:16-00:20

3.4 Using the **Slice Number** slider in the **Properties** section with the initial time point data showing the distal Ortho Slice, align the comparison week slice number to match the distal slice of the initial time point **[1]**. Note the slice number for the comparison week data set's distal slice and repeat for the proximal slice **[2]**.

3.4.1 SCREEN: 67904_Screenshot_03.mp4 . 00:37-00:49
3.4.2 SCREEN: 67904_Screenshot_04.mp4 . 00:30-00:40

3.5 Click on the initial time point for the extracted radius and in the **Properties** section, click on the **Crop Editor** tool **[1]**.

3.5.1 SCREEN: 67904_Screenshot_05.mp4 . 00:02-00:15

3.6 Within the **Crop Editor** pop-up, input the minimum and maximum values in the X, Y, or Z fields **[1]**. Observe the viewing window as the region of interest adjusts, then click **OK** to crop the data set **[2]**.

3.6.1 SCREEN: 67904_Screenshot_05.mp4 . 00:30-00:42

3.6.2 SCREEN: 67904_Screenshot_05.mp4. 00:42-00:46

3.7 Repeat the crop procedure for the comparison week data set **[1]**.

3.7.1 SCREEN: 67904_Screenshot_05.mp4 . 00:53-00:59 and 01:28-01:32

4. Volume Analysis of Data Sets

4.1 To determine the volume of the initial time point data set, right-click on the transformed initial time point data set for the extracted radius **[1]**, search for **Material Statistics**, and select it **[2]**. In the **Properties** section, set **Data** as the transformed initial time point data set, select **Materials**, and click **Apply** **[3]**.

4.1.1 SCREEN: 67904_Screenshot_06.mp4. 00:03-00:07

4.1.2 SCREEN: 67904_Screenshot_06.mp4. 00:07-00:12

4.1.3 SCREEN: 67904_Screenshot_06.mp4. 00:12-00:33

4.2 Click on the new **.MaterialStatistics** *(material statistics)* data set, then in the **Properties** window click on **Spreadsheet Show [1]**. Click the **Tables** tab above the window to view the volume of the cropped initial time point data set **[2]**.

4.2.1 SCREEN: 67904_Screenshot_06.mp4. 00:33-00:36

4.2.2 SCREEN: 67904_Screenshot_06.mp4. 00:36-00:43

4.3 Repeat the volume analysis steps for the comparison week data set **[1]** and then go to the **Tables** tab to view both data sets with separate volume tabs **[2]**.

4.3.1 SCREEN: 67904_Screenshot_06.mp4. 00:43-01:02

4.3.2 SCREEN: 67904_Screenshot_06.mp4. 01:02-01:13

5. Visualization of Data Sets

5.1 To visualize the change in bone volume, right-click on the comparison week transformed data set for the extracted radius, search for **Arithmetic**, and select it **[1]**.

5.1.1 SCREEN: 67904_Screenshot_07.mp4. 00:05-00:14

5.2 In the **Properties** window, set **Input A** as the comparison week transformed data set, **Input B** as the initial time point data set **[1]**, **Input C** as **No Source**, **Result Type** as

Input A, leave **Option** unchecked **[2]**, set **Result Channels** as like Input A, and set **Expression** as **A-B** *(A-B)* **[3]**.

5.2.1 SCREEN: 67904_Screenshot_07.mp4. 00:14-00:26
5.2.2 SCREEN: 67904_Screenshot_07.mp4. 00:26-00:30
5.2.3 SCREEN: 67904_Screenshot_07.mp4. 00:30-00:44

5.3 Click on the resulting data set and press **F2** to rename the file **[1]**. Then, right-click on this result data set, search for **Generate Surface**, and select it **[2]**. In the **Properties** window, click **Apply**, and in the pop-up window, click **Continue** to create a new **.surf** *(surf)* data set **[3]**.

5.3.1 SCREEN: 67904_Screenshot_07.mp4. 01:16-01:29
5.3.2 SCREEN: 67904_Screenshot_07.mp4. 01:29-01:42
5.3.3 SCREEN: 67904_Screenshot_07.mp4. 01:42-01:54

5.4 Right-click on the **.surf** data set, search for **Surface View**, and select it **[1]**. A surface view of the arithmetic result will appear in the viewing window **[2]**.

5.4.1 SCREEN: 67904_Screenshot_07.mp4. 01:54-02:02
5.4.2 SCREEN: 67904_Screenshot_07.mp4. 02:02-02:19

5.5 To change the color of the surface view, click on the surface view in the **Project View** window **[1]**. In the **Properties** window, open the **Colors** drop-down, select **Constant**, then click on **Colormap** and assign a preferred color **[2]**.

5.5.1 SCREEN: 67904_Screenshot_07.mp4. 02:19-02:24
5.5.2 SCREEN: 67904_Screenshot_08.mp4. 00:02-00:14

5.6 To view bone volume change on the initial week data set, right-click on the **.transformed** *(transformed)* data set, search for **Extract Label**, and select it **[1]**.

5.6.1 SCREEN: 67904_Screenshot_08.mp4. 00:25-00:40

5.7 In the **Properties** section, set **Labels** to the **.transformed** data set, **Label ID** to **2**, and check **Export to Binary**, then click **Apply** to generate a result data set **[1]**. Then, press **F2** to rename the result file **[2]**.

5.7.1 SCREEN: 67904_Screenshot_08.mp4. 00:40-00:53
5.7.2 SCREEN: 67904_Screenshot_08.mp4. 00:53-01:03

5.8 Right-click on the new result data set, search for **Generate Surface**, and select it **[1]**. In the **Properties** window, click **Apply**, and in the pop-up window, click **Continue** to create a new **.surf** data set **[2]**.

5.8.1 SCREEN: 67904_Screenshot_08.mp4. 01:03-01:07

5.8.2 SCREEN: 67904_Screenshot_08.mp4. 01:07-01:24

5.9 Next, right-click on the new **.surf** data set, search for **Surface View**, and select it. A surface view of the arithmetic result will appear **[1]**.

5.9.1 SCREEN: 67904_Screenshot_08.mp4. 01:24-01:30

5.10 To change the color of this surface view, click on the surface view in the **Project View** window **[1]**. In the **Properties** window, open the **Colors** drop-down, select **Constant**, then click on **Colormap** and assign a preferred color **[2]**.

5.10.1 SCREEN: 67904_Screenshot_08.mp4. 01:24-01:31

5.10.2 SCREEN: 67904_Screenshot_08.mp4. 01:31-01:45

Results

6. Results

6.1. Micro-CT images of three unique rat models, each treated with a polycaprolactone scaffold for 6 weeks, were investigated. Solid models from weeks 0 and 6 were successfully aligned using shared anatomical regions, enabling direct longitudinal comparison **[1]**, and a merged model was generated to confirm registration accuracy **[2]**.

6.1.1. LAB MEDIA: Figure 2. *Video editor: Highlight the top and middle rows labelled "WK 0" and "WK 6".*

6.1.2. LAB MEDIA: Figure 2. *Video editor: Highlight the bottom row "WK 0+6"*

6.2. Subtracting the week 0 region of interest from the week 6 region of interest **[1]** revealed a distinct 3D model of bone volume change within the defect site **[2]**.

6.2.1. LAB MEDIA: Figure 3. *Video editor: Highlight the middle and left segments labelled "WK 6 ROI" and "WK 0 ROI".*

6.2.2. LAB MEDIA: Figure 3. *Video editor: Highlight the far-right segment labelled "Bone Change ROI"* .

6.3. Visual overlays of bone volume changes from week 0 to week 6 demonstrated variability across triplicate trials for each polycaprolactone scaffold **[1]**, with consistent defect bridging evident in PCL 2 models **[2]**.

6.3.1. LAB MEDIA: Figure 4. *Video editor: Sequentially highlight each column*

6.3.2. LAB MEDIA: Figure 4. *Video editor: Zoom in on the PCL 2 row*

Pronunciation guide :

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1. Diaphysis

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/diaphysis
- **IPA**: /daɪˈæfəsɪs/
- **Phonetic Spelling**: dye-AF-uh-sis(merriam-webster.com, merriam-webster.com)

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2. Voxel

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/voxel
- **IPA**: /ˈvɒksəl/
- **Phonetic Spelling**: VOK-suhl

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3. Interpolation

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/interpolation
- **IPA**: /ɪnˌtɜːrpəˈleɪʃən/
- **Phonetic Spelling**: in-TUR-puh-LAY-shun(merriam-webster.com, merriam-webster.com)

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4. Transverse

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/transverse
- **IPA**: /ˈtrænsvɜːrs/
- **Phonetic Spelling**: TRANS-vers(merriam-webster.com, merriam-webster.com)

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5. Proximal

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/proximal
- **IPA**: /ˈprɒksɪməl/
- **Phonetic Spelling**: PROK-sih-muhl(merriam-webster.com, merriam-webster.com)

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6. Distal

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/distal
- **IPA**: /ˈdɪstəl/
- **Phonetic Spelling**: DIS-tuhl

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7. Resample

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/resample

- **IPA**: /riːˈsæmpəl/
- **Phonetic Spelling**: ree-SAM-puhl(merriam-webster.com, merriam-webster.com)

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8. Rigid

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/rigid
- **IPA**: /ˈrɪdʒɪd/
- **Phonetic Spelling**: RIJ-id

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9. Correlation

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/correlation
- **IPA**: /ˌkɔːrəˈleɪʃən/
- **Phonetic Spelling**: kor-uh-LAY-shun(merriam-webster.com)

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10. Transformation

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/transformation
- **IPA**: /ˌtrænsfərˈmeɪʃən/
- **Phonetic Spelling**: trans-fer-MAY-shun(merriam-webster.com)

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11. Alignment

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/alignment
- **IPA**: /əˈlaɪnmənt/
- **Phonetic Spelling**: uh-LINE-ment

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12. Arithmetic

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/arithmetic
- **IPA**: /əˈrɪθmətɪk/
- **Phonetic Spelling**: uh-RITH-muh-tik

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13. Scaffold

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/scaffold
- **IPA**: /ˈskæfəld/
- **Phonetic Spelling**: SKAF-uhld

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14. Registration

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/registration
- **IPA**: /ˌrɛdʒɪˈstreɪʃən/
- **Phonetic Spelling**: rej-ih-STRAY-shun
