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Title: Quantitative Assessment Protocol for Facial Soft Tissue Volumetric Changes with Stereophotogrammetry

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Author Questionnaire

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

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

3. **Filming location:** Will the filming need to take place in multiple locations? **NO**

4. **Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **NO**

Current Protocol Length
Number of Steps: 16
Number of Shots:34

Introduction

INTRODUCTION:

~~What is the scope of your research? What questions are you trying to answer?~~

1.1. **Ermina Lee:** Our protocol provides a methodology for the conduction of a semi-automated, high-accuracy and high reproducibility 3D volumetric change assessment using facial surface imaging.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~What technologies are currently used to advance research in your field?~~

1.2. **Ermina Lee:**3D imaging enables quantitative and comprehensive assessment of craniofacial morphology, growth and development, and pathology, surpassing the limitations of traditional 2D techniques.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What advantage does your protocol offer compared to other techniques?~~

1.3. **Ermina Lee:** Prior facial soft tissue assessment methods require extensive training in specialized image analysis software, multiple imaging modalities and software, or extensive landmarking. We present here a straightforward approach that does not require expertise in the field of image analysis.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~How will your findings advance research in your field?~~

1.4. **Ermina Lee:** This is a practical guide for clinical assessment and research evaluating the impact of therapeutic interventions on facial aesthetics and soft tissues. This type of assessment is applicable to fields of dentistry, and particularly fixed and removable prosthodontics, orthodontics, and orthognathic surgery.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~What questions will future research focus on?~~

1.5. **Ermina Lee:** This methodology is expected to be used in the future for the deeper understanding of soft tissue response to different treatment modalities. This knowledge will enable customized treatment approaches and the optimization of treatment results.

1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

This research has been approved by the Institutional Review Board (IRB) at the National Institutes of Health

2. Baseline Facial Image Registration to the 3D Axis Grid

Demonstrator: Ermina Lee

2.1. To begin, open the 3D facial photograph on the imaging software **[1-TXT].** Show the axis grids in the viewport **[2]**. Click **Snap View** to square the image to the nearest ninety-degree frontal view **[3]**.

2.1.1. WIDE: Talent opening the image on the imaging software.
TXT: Texture only images were used to protect the identity of the subject

2.1.2. SCREEN: 69204_screenshot_2.mp4. 00:00-00:04

2.1.3. SCREEN: 69204_screenshot_2.mp4. 00:05-00:07

2.2. From the left-side menu, select **Spin Active Surfaces** icon and rotate the image until it is evenly bisected by the vertical Y axis **[1]**. Now, use the **Paint Area Selection** tool and drag the brush across the frontal surface of the image to highlight the face **[2]**.

2.2.1. SCREEN: 69204_screenshot_2.mp4 00:08-00:23

2.2.2. SCREEN: 69204_screenshot_2.mp4 00:24-00:48

2.3. Once the region is selected, click **Find Symmetry** to automatically align the image along the vertical axis through its center **[1]**. Use **Clear Area** to deselect the region after selection **[2]**.

2.3.1. SCREEN: 69204_screenshot_2.mp4 00:49-00:54.

2.3.2. SCREEN: 69204_screenshot_2.mp4 00:55-00:59

2.4. To correct image rotation and establish front-to-back orientation for registration, begin by displaying the image in two viewports **[1]**. Use the **Spin tool** and **Snap** function in one viewport to obtain a lateral view **[2]**.

2.4.1. SCREEN: 69204_screenshot_2.mp4 01:00-01:02

2.4.2. SCREEN: 69204_screenshot_2.mp4 01:03-01:11.

2.5. In the lateral viewport, use **Roll Active Surfaces** to adjust the image so the head is aligned vertically with the grid **[1]**, then use **Pan Active Surfaces** to center the image on the main vertical axis **[2]**. Save the registered image **[3]**.

2.5.1. SCREEN: 69204_screenshot_2.mp4 01:12-01:22

2.5.2. SCREEN: 69204_screenshot_2.mp4 01:23-01:38.

2.5.3. SCREEN: 69204_screenshot_2.mp4 01:39-01:40

3. Landmark Annotation, Region Selection, and Registration of Subsequent Images for Volume Measurement

3.1. To annotate landmarks, use the **Landmark** option from the left side menu **[1]**.

3.1.1. SCREEN: 69204_screenshot_3.mp4 00:00-00:02

3.2. Annotate the **Medial canthus** or **Endocanthion** bilaterally ~~Place the left first, then the right~~, verifying correct placement in the frontal view . Then annotate **Lateral canthus** or **Exocanthion** in the same order. Annotate **Glabella** by finding the most anterior midpoint on soft tissue contour **[1]**.

3.2.1. SCREEN: 69204_screenshot_3.mp4 00:03-00:36.

~~3.2.2. SCREEN: Place the landmarks for the lateral canthus and verify in the frontal view.~~

~~3.2.3. SCREEN: The most anterior midpoint on the soft tissue contour is being found and marked in left and right view. Then the midline is being seen in the frontal view.~~

3.3. Save the landmarked image **[1]**.

3.3.1. SCREEN: The landmarked image is being saved. 00:37-00:42

3.4. Next, in the frontal view, select **Pick Multiple Points for Closed Loop** and place points along the perimeter of the region of interest **[1]**. After finishing the loop, select **Add to Area [2]**. ~~Then use the **Paint Area** tool to refine selection if needed **[3 TXT].**~~

3.4.1. SCREEN: 69204_screenshot_4.mp4. 00:00-00:27

3.4.2. SCREEN: 69204_screenshot_4.mp4 02:28-00:32

~~3.4.3. SCREEN: The Paint Area tool is being used to refine selection. **TXT: Repeat in lateral and submental views**~~

~~3.5. Once satisfied, use **Copy Area** to create a mask **[1].** Apply **Closed Surface Volume** tool to measure the total volume within the region of interest **[2].** Save the image mask **[3].**~~

~~3.5.1. SCREEN: **Copy Area** is being used to create a mask.~~

~~3.5.2. SCREEN: **Closed Surface Volume** is being used to measure the total volume within the ROI.~~

~~3.5.3. SCREEN: The image mask is being saved.~~

3.6. Now, open both the baseline image and the subsequent image to be registered or registration image **[1]**. ~~Select two **Side-by-Side** viewport view with synchronization toggled off **[2].**~~

3.6.1. SCREEN: 69204_screenshot_5_new.mp4 00:00-00:05.

~~3.6.2. SCREEN: The **Side-by-Side** layout is being selected then the sync is being toggled off.~~

3.7. ~~Use the **Spin tool** to manually rotate the registration image until its orientation closely matches the baseline image **[1].**~~ Place the landmarks in the same sequence as before **[2]**.

~~3.7.1. SCREEN: The new image is being rotated using the Spin tool.~~

3.7.2. SCREEN: 69204_screenshot_5.mp4 00:06-00:11, 00:42-00:43.

3.8. Select **Register Surfaces** then move the registration **image** to fit the surface **baseline** using **landmarks with corresponding names [1]**.

3.8.1. SCREEN: 69204_screenshot_5.mp4 00:44-00:54.

3.9. Switch to a single viewport and check registration using the **Color Surface by Distance tool [1].** Select **color whole of** and choose **registration** image from the **this surface** options and the **baseline** image from the **by the distance to this surface** options. Click on Calculate distances to generate a distance-based color heat-map **[2]**.

3.9.1. SCREEN: 69204_screenshot_5.mp4 00:55-01:09.

3.9.2. SCREEN: 69204_screenshot_5.mp4 01:10-01:26.

3.10. Return to two viewports, toggle off the synchronisation, and hide the registration image **[1]**. Use **Project Selected Area** to highlight the corresponding region from baseline onto the registration image **[2]**. Select the **Reverse surface color** from the Preferences menu **[3].** Then select **Hide** from the Area menu to hide the selected area from the baseline image **[4]**. Click on the registration image and then select the **Between Two Surfaces option** from the Measure Volume options. **[5]**.

3.10.1. SCREEN: 69204_screenshot_5.mp4 01:27-01:43

3.10.2. SCREEN: 69204_screenshot_5.mp4 01:44- 01:53.

3.10.3. SCREEN: 69204_screenshot_5.mp4 01:54-02:01

3.10.4. SCREEN: 69204_screenshot_5.mp4 02:02-02:07

3.10.5. SCREEN: 69204_screenshot_5.mp4 02:08-02:22

3.11. Now return to one viewport view and hide the registration image **[1]**. Select the **+/- Volumes** option from the Surface menu **[2]** and then measure the volume between the two selected areas by using the **Volume Of Closed Surface** option from the Measure menu **[3]** . ~~The results can be found in the Log area at the bottom of the window. **[1].** Save the registered image with volume annotation **[2].**~~

3.11.1. SCREEN: 69204_screenshot_5.mp4 02:23-02:29

3.11.2. SCREEN: 69204_screenshot_5.mp4 02:30-02:40

3.11.3. SCREEN: 69204_screenshot_5.mp4 02:41-02:59

Results

4. Results

4.1. Baseline three-dimensional facial images were acquired one year after surgery and registered using anatomical landmarks including the bilateral medial and lateral canthi and the glabella **[1].** Postoperative images captured one week after surgery were registered to the same axis grid, with the baseline region of interest projected onto them **[2]**.

4.1.1. LAB MEDIA: Figure 1. *Video editor: Please highlight Image A*

4.1.2. LAB MEDIA: Figure 1 *Video editor: Please highlight Image B*

4.2. Baseline and postoperative images were superimposed to verify the accuracy of alignment **[1]**. Volumetric masks from both time points were generated based on the region of interest and used to calculate the volume in cubic millimeters **[2]**.

4.2.1. LAB MEDIA: Figure 1 *Video editor: Please highlight Image C*

4.2.2. LAB MEDIA: Figure 1 *Video editor: Please highlight Image D*

4.3. A heat map was produced to compare volume differences between the masks, showing increased volume in blue and green and decreased volume in yellow and orange **[1]**.

4.3.1. LAB MEDIA: Figure 1 *Video editor: Please highlight Image E*

Pronunciation Guide:
Quantitative
Pronunciation link: https://www.merriam-webster.com/dictionary/quantitative
IPA: /ˈkwɑːntəˌteɪtɪv/ , /ˈkwɑːntɪˌteɪtɪv/
Phonetic Spelling: kwon-tuh-tay-tiv
Volumetric
Pronunciation link: https://www.merriam-webster.com/dictionary/volumetric
IPA: /ˌvɑːljəˈmɛtrɪk/
Phonetic Spelling: vol-yuh-meh-trik
Stereophotogrammetry
Pronunciation link: No confirmed link found
IPA: /ˌstɛrioʊˌfoʊtəˈgræmɪtri/
Phonetic Spelling: stair-ee-oh-foh-tuh-gram-ih-tree
Craniofacial
Pronunciation link: https://www.merriam-webster.com/dictionary/craniofacial
IPA: /ˌkreɪnioʊˈfeɪʃəl/
Phonetic Spelling: kray-nee-oh-fay-shul
Morphology
Pronunciation link: https://www.merriam-webster.com/dictionary/morphology
IPA: /mɔːrˈfɑːlədʒi/
Phonetic Spelling: mor-faa-luh-jee
Pathology
Pronunciation link: https://www.merriam-webster.com/dictionary/pathology
IPA: /pəˈθɑːlədʒi/
Phonetic Spelling: puh-thaa-luh-jee
Reproducibility
Pronunciation link: https://www.merriam-webster.com/dictionary/reproducibility
IPA: /ˌriːprəˌduːsəˈbɪləti/
Phonetic Spelling: ree-pro-doo-suh-bil-uh-tee
Prosthodontics
Pronunciation link: https://www.merriam-webster.com/dictionary/prosthodontics
IPA: /ˌprɑːsθəˈdɑːntɪks/
Phonetic Spelling: pross-thuh-don-tiks
Orthodontics
Pronunciation link: https://www.merriam-webster.com/dictionary/orthodontics
IPA: /ˌɔːrθəˈdɑːntɪks/
Phonetic Spelling: or-thuh-don-tiks
Orthognathic
Pronunciation link: No confirmed link found
IPA: /ˌɔːrθəgˈnæθɪk/
Phonetic Spelling: or-thug-nath-ik
Canthus
Pronunciation link: https://www.merriam-webster.com/dictionary/canthus

IPA: /ˈkænθəs/
Phonetic Spelling: kan-thus
Endocanthion
Pronunciation link: No confirmed link found
IPA: /ˌɛndoʊˈkænθiˌɑːn/
Phonetic Spelling: en-doh-kan-thee-on
Exocanthion
Pronunciation link: No confirmed link found
IPA: /ˌɛksoʊˈkænθiˌɑːn/
Phonetic Spelling: ek-soh-kan-thee-on
Glabella
Pronunciation link: https://www.merriam-webster.com/dictionary/glabella
IPA: /gləˈbɛlə/
Phonetic Spelling: gluh-bell-uh
Submental
Pronunciation link: https://www.merriam-webster.com/dictionary/submental
IPA: /ˌsʌbˈmɛntəl/
Phonetic Spelling: sub-men-tul