3D Strain Analysis Protocol

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This protocol will take you through all of the necessary steps to perform 3D tracking of beads using images taken with FujiFilm 3D W3 Stereovision Camera.

1. Folder Structure

* See below flow chart for how folders should be structured for code to run properly.
* Blocks with colored in backgrounds are folders, outlined blocks are groups of files.
* Orange block indicates the “local” or parent folder containing all of the data, codes, analysis, etc. required for 3D Strain analysis. The name of this folder doesn’t matter.
* The blue blocks should not change. These will contain all of the necessary codes required for smooth analysis as outlined below.
* User needs to create green folders for each experiment.
  + An “experiment” is defined as a group of images or videos that the user wants to group together for convenience. This can be all videos from a particular date, a set of experimental videos, etc.
* A screenshot of a cell phone

  Description automatically generatedMATLAB will name File Name folders & all contained files based on the file name of the 3D video selected. ie. DSCF 7777. If you want the folder to be more specific, the file name within 3D must be changed.

1. Image Acquisition Notes

* User will need to take an image of the field of view with the calibration cylinder in the same plane as the points of interest for proper camera calibration.
  + The calibration cylinder must be vertical to allow for proper calibration.
  + Calibration information will be included in part D of this protocol.
* It is important to record what zoom setting images are acquired with, and calibrate the field of view on that zoom setting
* Put the original images you take into Local>Experiment>Video or Image>3D.

1. Image Splitting (Resolving 3D Images into Left and Right Views)

* This step will take the original 3D images or video that you take with the camera and resolve them into left and right components.
  + 3D original images/videos should be saved in:
    - **Local>Experiment>Video or Image>3D**
  + Corresponding images/videos that have been split should be saved in:
    - **Local>Experiment>Video or Image>Split**
* Methods of splitting images and videos:
* Mac using High Sierra or Earlier:
  + StereoSplicer 🡪 simple to use, but results in loss of quality of image.
  + Otherwise:

Open up a terminal window and run the following two lines of code with the correct path/file name replacing the red text.

***Video***

*Update 8Nov2020*

*I created a shell script to make this a little easier on Linux/Macs. It is called splitMe.sh and is in the Matlab Code folder. To use this:*

* + - 1. *Copy splitMe.sh to a Experiments>ExperimentName>Video or Image folder*
      2. *Open up splitMe.sh in Atom or notepad or some other text editor*
      3. *edit the file name to be the one you want to split*
      4. *go to the command line and navigate to the folder that splitMe.sh is in*
      5. *type ./splitMe.sh*
         * *IF you get a permission error type in:*

*Right click on split Me, get info*

*Scroll to sharing and permissions*

*Make sure all are “read and write”*

*chmod +x splitMe.sh*

* + - 1. *Repeat steps 2-5 for every video you want to split.*

ffmpeg -i **path to 3D file** -map 0:0 -map 0:1 -vcodec copy -acodec copy **path to new file to be created**

ffmpeg -i **path to 3D file** -map 0:2 -vcodec copy -acodec copy **path to new file to be created**

**Example:**

**These two lines of code take video DSCF7343 that is located on the desktop and splits into left and right videos to be saved also on the desktop.**

ffmpeg -i /Users/leanneiannucci/Desktop/DSCF7343.AVI -map 0:0 -map 0:1 -vcodec copy -acodec copy /Users/leanneiannucci/Desktop/DSCF7343\_L.AVI

ffmpeg -i /Users/leanneiannucci/Desktop/DSCF7343.AVI -map 0:2 -vcodec copy -acodec copy /Users/leanneiannucci/Desktop/DSCF7343\_R.AVI

***Photo***

Mungempo -m split \*.MPO

*This will split the photo into \_L.JPG and \_R.JPG*

\*\*\* I have not tested this as of 17Mar2020.

* This is not mandatory, but if you are analyzing videos after this point I **highly** recommend you TRIM YOUR VIDEOS. The longer your video, the longer the analysis code will take to complete. (I recommend doing this on a mac. I haven’t tested it on a PC as of 8Nov2020). To do this, you have to open up your \_L.AVI and \_R.AVI in quicktime (the default program, usually) and then trim them down. **It is very important that they are the exact same length (ie. number of frames). Matlab will not let you analyze it if the videos are different lengths.** Save them after they are trimmed with the same name as they wre before. They will be .mov files now, but this is OK.

1. Camera Calibration
   1. Open up a new MATLAB instance. Navigate directory to local folder. Type the following command into the matlab window.

addpath(genpath(cd))

* 1. Run the first step of the calibration process
     1. Before running this, it is important to have the images that will be used in the calibration in their proper location & labelled correctly. The left image should be appended with \_01 and the right image should be appended with \_02 in order for the code to work properly.
     2. Type the follow command into the matlab window:

STEP1\_CalcDLTparameters

* + 1. Select what type of computer you are using
    2. Navigate to images that will be used for calibration, select both in the dialog box on the left and then hit add. They should be added to the box on the right. Hit done.
    3. Dialog box will appear asking if you want to save DLT parameters. Hit yes.
    4. Navigate to folder where you want them to be saved.
    5. Navigate to and select the MAT file containing the “real world” coordinates of your calibration object. The cylinder made by griffin on 12Mar2020 has a corresponding file with the following name:

myCylinderCoordinates\_LEI\_12Mar2020.mat

* + - 1. If you are using a different calibration object, you will have to make a new file for this. This can be performed by typing

generate\_cylindrical\_calibration\_object\_coordinate\_file

into the command window. See the MultiDIC documentation for more details about this.

* + 1. A window with the first view will pop up. Draw a polygon around the region of interest. This ROI is a box containing a subset of columns in view. I usually select only the clearest ones, and not ones that are completely on the side of the cylinder. Double click on the polygon whne done. The mask will appear on the image, click the image to continue. Identify if your masking is correct.
    2. Enter the number of the columns your ROI contains. The “first” is your leftmost column and the “last” is your rightmost column.
    3. Adjust the grayscale threshold until all points are centered within yellow boxes and there is a blue cross in the center of each one and nowhere else. Hit finish when done. Indicate if centroids are okay.
    4. A close up of a device

       Description automatically generatedThe “sorted” points will appear on the screen overlayed on your image. These should be numbered top to bottom, left to right, starting in the top leftmost corner (see right image). See the MultiDIC documentation if this is unclear. If this is not the case either (a) redo the calibration until this point or (b) retake the images. Problems can arise most commonly if (a) there is a shadow cast over the cylinder and you can’t threshold well or (b) the cylinder is not lined up vertically with respect to the camera. If the cylinder is tilted **the calibration will not work**. If the points are okay, click on image to proceed.
    5. Repeat steps viii-xi with the other image.
    6. A dialog box saying step 1 is completed will appear.
  1. Run the second step of the calibration process
     1. Type the following into the command window

STEP1p\_DLTreconstruction

* + 1. Indicate what kind of computer you are running.
    2. Navigate to and select the DLT structures that you just generated from the last part of the calibration process. These should be located in the folder you selected in 2vi. They will be named DLTstruct\_cam\_1.mat and DLTstruct\_cam\_2.mat. Once they are added to the right box, hit done.
    3. Hit **yes** on the dialog box asking if you want to save DLT reprojection structure. Navigate to where you want it to be saved. This is the structure you will use in image analysis.
    4. Enter the indices of pairs of cameras into dialog box. If you did everything as directed up until this point, just hit OK on the dialog box.
    5. Hit **no** on the dialog box asking if you want to remove distortion from calibration points.
       1. See MultiDIC documentation for more information on this if you are interested.
    6. Lots of figures of the reconstruction & a box will appear and you are done with the calibration!

1. Image Analysis
   1. Type the following in the command window:

Coordinate3DCalculator\_v3\_macs

* 1. Select “local” folder.
  2. Select all 3D Videos and Images you want analyzed. These should be in **Local>Experiment>Video or Image>3D**
  3. Indicate whether you are using a mac or PC.
  4. Select correct camera parameters for video or image using analyzed. These should be located where you saved them in step D3iii and will be named DLTstructpairs.mat
  5. Indicate whether the file to be analyzed is an image or a video.
  6. Select left image/video file (**Local>Experiment>Video or Image>Split)**
  7. Select right image/video file (**Local>Experiment>Video or Image>Split)**
  8. If this is a video, wait for the video to be split into individual images. This will take a few minutes if the video is large.
  9. It will ask if you want to perform color thresholding. In all likelihood the answer here is **no**.
  10. The strain tracking function will load for the left file first. If it is a video that you input in, the first frame will be loaded in the thresholder.
  11. Move the scroll bars left and right such that the dots you are looking to track are white and they are properly isolated from the surrounding black background. These threshold values will be used to threshold all of the images in the video so it important to have good contrast between points and background. Press Done when finished.
  12. Type into the dialog box the number of markers that you are looking to track and hit okay. Remember/record the order in which you select these markers since you will need that later. A second dialog box will appear telling you to click one time on each marker. Hit OK on that box and then select each marker you wish to track one at a time.
  13. The image will be converted to grayscale and red circles will appear on what the program identifies as the center of each marker. This process will repeat for each image of the video. After all images have been marked, the program will ask you if you are satisfied with tracking. If all markings seemed to be accurate, press yes. If not, select “No, I want to adjust”.
  14. Repeat steps 11-14 for the right file.
  15. MAT file with the name of the 3d image/video file will be saved in 3D Strain>Experiment Folder>Data>File Name.