**Protocol for DVC Correlation:***DM, last updated: 05/01/18*

* Start with the enhanced virtual tiff files exported by FIJI.
Once you have all the files enhanced, open matlab:
	+ Copy the matlab file: **convert\_multitiff\_to\_mat.m** into the directory with the images and set that as the new folder directory in matlab. Enter the name of each multitiff file, and click “Run” to convert each .tiff file to a .mat file in matlab
	+ Copy the resulting .mat files to the FIDVC directory:
	“FIDVC Human LC-PPS SHG&TPF LSM Rectangular Volume”
	+ Load the script **Virtual\_Displacement.m**. Input the filename of the 5 mmHg, Set 2 volume as your reference volume and the 5 mmHg, Set 1 as your warped volume in the name sections. Run the file. It will return images of the new reference and warped images simulating a triaxial strain state and displacement and save then as new .mat files with \_ref and \_warp after the name. Do this for both the SHG and TPF channels. This will be used as a DVC test to identify the correlation errors across the volume and decide which areas can accurately correlate for this volume.
	+ Now, set up the names of the volumes you want to run in DVC and open up the **Sequential\_Runs.m** matlab file from the DVC directory to input the names in the order you want to correlate them. This can be adjusted based on what data you have but here is the suggested protocol for a standard 3-pressure run:
		- DVC loads files as sets of names. It will look for a particular name with a varying number. The varying number should be 1 and 2 to show DVC that “1” is the reference volume and “2” is the deformed volume. I use letters followed by a number to indicate the run type and the reference and deformed volumes. Instructions for queuing multiple DVC runs are included in the **Sequential\_Runs\_template.m** file. Some volumes may need to be copied and renamed to queue all 4 runs at once:
		- A. Baseline Error DVC Correlation: rename the 5 mmHg, Set 2 volume so that it includes “\_aX\_” after the LE/RE part of the name, Example:

		1HLC\_H15\_120817\_RE\_a1-05mmHg\_Set1\_Stitch\_SHG\_decon-3DCE.mat
		1HLC\_H15\_120817\_RE\_a2-05mmHg\_Set2\_Stitch\_SHG\_decon-3DCE.mat
		- B. Correlation Error DVC Correlation: rename the 5 mmHg, Set 2 reference and warped volumes so they include “\_bX\_” after the LE/RE part of the name, Example:

		1HLC\_H15\_120817\_RE\_b1-05mmHg\_Set2\_Stitch\_SHG\_decon-3DCE\_ref.mat
		1HLC\_H15\_120817\_RE\_b2-05mmHg\_Set1\_Stitch\_SHG\_decon-3DCE\_warp.mat
		- C. Real Displacement 5🡪10 mmHg: copy and rename the 5 mmHg, Set 2 volume and 10 mmHg Set 2 volume“\_cX\_” after the LE/RE part of the name, Example:
		1HLC\_H15\_120817\_RE\_c1-05mmHg\_Set2\_Stitch\_SHG\_decon-3DCE.mat
		1HLC\_H15\_120817\_RE\_c2-10mmHg\_Set2\_Stitch\_SHG\_decon-3DCE.mat
		- C. Real Displacement 10🡪45 mmHg: copy and rename the 10 mmHg, Set 2 volume and 45 mmHg Set 2 volume“\_cX\_” after the LE/RE part of the name, Example:
		1HLC\_H15\_120817\_RE\_d1-10mmHg\_Set2\_Stitch\_SHG\_decon-3DCE.mat
		1HLC\_H15\_120817\_RE\_d2-45mmHg\_Set2\_Stitch\_SHG\_decon-3DCE.mat
		- For a 5-pressure run the additional 2 incremental correlations should be named as “\_eX\_” and “\_fX\_” for:
		5🡪7.5 mmHg and 10🡪25 mmHg.
		- Directions and formatting are provided in for you for a 3-pressure run in **Sequential\_Runs\_template.m**: You can copy the 3 lines that call DVC as many times as you like and set the filenames and desired number of iterations to queue as many runs as you like. In brief: copy the part of the name up to the letter, but not with the number or past into Sequential\_Runs.m. Put an ‘\*’ after the letter to indicate to DVC to look for files with that name and some number afterward. Make sure only two files in your DVC directory (the reference and deformed) have this format for a name.
		- Run Sequential\_Runs.m on a server. It will take about a day to finish all 4 correlations completely, and will result in the 4 result files named: resultsFIDVC-filenameA.mat, resultsFIDVC-filenameB.mat…etc. However many you queued.
		- After it is finished, rename your files, noting the SHG or TPF, which two volumes were correlated, the calculation spacing, and the kind “Displacement”, “Correlation Error”, or “Baseline Error”, suggested naming conventions are shown below:

		resultsFIDVC-H15-120817-RE-SHG-Baseline-45mmHg-884.mat
		resultsFIDVC-H15-120817-RE-SHG-corrError-05mmHg-442.mat
		resultsFIDVC-H15-120817-RE-SHG-Disp-05-10mmHg-442.mat
		resultsFIDVC-H15-120817-RE-SHG-Disp-10-45mmHg-442.mat
		- Run the above analysis again, identically, for the TPF volumes after the SHG volumes
		- To convert the incremental correlations of 5🡪10 and 10🡪45 mmHg or 5🡪10 and 10🡪25 mmHg into a cumulative displacement correlation between 5🡪25 mmHg and 5🡪45 mmHg use the program:
		**cumulativeDisplacementFromIncrementalCorrelations.m**
		- Directions for use are included in this file. In brief: you need to enter the saved filename of the first DVC pressure increment and the second pressure increment, tell the program what the calculation spacing was (4-4-2), and how many pressure increments there are (should only be 2). Once these are entered, click “Run” and the cumulative displacement field and calculation point positions will be calculated. It will save automatically as “filename2\_cum.mat”. You can rename it as you wish.
		- DVC results will already be unpackaged for this run, so you do not need to use the DVC cell unpackaging function when processing cumulative runs later.

**Protocol for Displacement Processing and Strain Calculation:**

*DM, last updated: 05/01/18*

* Start with the resultsFIDVC-filename.mat correlation results exported by **Sequential\_Runs.m** after DVC correlation. These files will have the following saved variables:

u – a cell data structure containing the x,y,z displacements between the two volumes inputted in the Sequential\_Runs.m file
sSize – shows the convergence of the subset size at every iteration. This should be 128x128x64 🡪 64x64x32
sSpacing – shows the convergence of the calculation spacing at every iteration. This should be 32x32x16 🡪 4x4x2
SSE – shows the sum of squared error at every iteration. For a quality volume this is generally less then 0.3 at the last iteration, but for noisier volumes it will be higher
dm – the final calculation spacing
cc – a cell data structure containing the correlation coefficients for every displacement calculation in the volume

*Refer to the Lab wiki for the rest of this protocol.*