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TITLE: Simplified protocol for meso-scale particle image velocimetry studies of neurovascular flows ~~*in vitro*~~*in vitro*

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KEYWORDS:
Particle Image Velocimetry, PDMS Tissue Phantom, 3D Printing, Fluid Mechanics, Signal Processing, Neurovascular.

SUMMARY:
Within this protocol we detail simplified methods for fabricating transparent neurovascular phantoms and characterizing flow therein. We highlight several important parameters and demonstrate their relationship to field accuracy.

ABSTRACT:
Particle Image Velocimetry (PIV) is used in a wide variety of fields, due to the opportunity it provides for precisely visualizing and quantifying flows across a large spatiotemporal range. However, its implementation typically requires use of expensive and specialized instrumentation, which limits its broader utility. Moreover, within the field of bioengineering, *in vitro* flow

visualization studies are also often further limited by the high cost of commercially-sourced tissue phantoms that recapitulate desired anatomical structures, particularly for those that span the meso-scale regime (i.e., sub-mm to mm length-scales). Herein, we present a simplified experimental protocol developed to address these limitations, the key elements of which include: 1) a relatively low-cost method for fabricating meso-scale tissue phantoms using 3D printing and silicone casting; and 2) an open-source image analysis and ~~pre/post-~~processing framework that reduces demand upon the instrumentation for measuring meso-scale flows (i.e., velocities up to tens of mm/s). Collectively, this lowers the barrier to entry for non-experts by leveraging resources already at the disposal of many bioengineering researchers. ~~, thus circumventing the~~ applicability of this protocol within the context of neurovascular flow characterization; however, it is expected to be relevant to a broader range of meso-scale applications in bioengineering and beyond.

INTRODUCTION:

Particle image velocimetry (PIV) is widely used in experimental fluid mechanics for flow visualization and quantitative investigations of fluid motion that vary in length-scale from atmospheric to microcirculatory flows[1–3]. While the specifics of its implementation can vary as widely as its applications, one aspect common to nearly all PIV studies is the use of video imaging of tracer particles seeded within the working fluid, followed by pair-wise analysis of consecutive image frames to extract desired flow characteristics. Typically, this is accomplished by first sub-dividing each image frame into smaller regions termed interrogation windows. As a consequence of the random positions of the dispersed particles, each interrogation window contains a unique distribution of pixel intensities. If the window size and data acquisition rate are chosen appropriately, cross-correlation of the intensity signal in each window can be used to estimate the average displacement within that region. Finally, given that the magnification and frame rate are known experimental parameters, an instantaneous velocity vector field can be readily computed.

A major advantage of PIV over single-point measurement techniques is its ability to map vector fields across a two- or three-dimensional domain. Hemodynamic applications, in particular, have benefited from this capability, since it allows a thorough investigation of local flows, which are known to play a significant role in vascular disease or remodeling (e.g., atherosclerosis, angiogenesis)[4–6]. This has also been true for the evaluation of neurovascular flows, and the interactions thereof with endovascular devices (e.g., flow diverters, stents, intra-saccular coils, etc.), since the relevant lengths-scales in such applications can often span one or more orders of magnitude (e.g., micrometer to mm-scale), and device geometry and placement can significantly impact the local fluid mechanics[7].

Most groups conducting PIV-based hemodynamics studies have relied on experimental setups that closely mimic some of the earliest investigations of stent influence on vascular flow[7, 8]. Typically, these include: a) pulsed lasers and high-speed cameras, to capture high velocity flows; b) synchronizers, to prevent aliasing between the pulse frequency of the laser and the camera acquisition frame rate; c) cylindrical optics, to form a light sheet, and thus, minimize background fluorescence from tracer particles above and below the interrogation plane; and d) in the case of

commercial turn-key systems, proprietary software packages, to perform the cross-correlation analyses. However, while some applications require the performance and/or versatility collectively afforded by these components, many others do not~~, thus making it sometimes difficult to justify the high cost of such systems. (which can reach up to > $100k)~~. ~~-~~Moreover, the high cost of commercially-sourced tissue phantoms that recapitulate desired vascular structures can also prove limiting for many ~~*in vitro*~~*in vitro* studies, particularly for phantoms with features that bridge the meso-scale regime (> $500/phantom). ~~It must be emphasized that the intent of this protocol is to outline a methodology to perform PIV with lab components that are already accessible to bioengineering laboratories. It is out of the scope of this document to provide a detailed guide on PIV and the reader is referred to comprehensive texts for variations on the method (CITE). Here, we lay out a fail-proof framework that can be readily applied even if one lacks experience with this measurement technique.~~ Herein, we report the development of a simplified~~e and low cost~~ protocol for implementing PIV for *in vitro* visualization of neurovascular flows, which typically lie both spatially and temporally within the meso-scale regime (i.e., length-scales ranging from sub-mm to mm, and velocities up to tens of mm/s). The protocol seeks to leverage resources already at the disposal of many bioengineering researchers, thus lowering the barrier to entry for non-experts.

~~It must be emphasized that the intent of this protocol is to outline a methodology to perform PIV~~ in-house fabrication of transparent, polydimethylsiloxane (PDMS)~~silicone~~-based tissue phantoms from 3D-printed sacrificial ~~acrylonitrile butadiene styrene (ABS)~~ molds. By leveraging the increasing availability of 3D printers in recent years, particularly those in shared/multi-user facilities (e.g., institutional facilities or public makerspaces), this methodology cuts cost significantly (e.g., < $100/phantom in our case), while enabling rapid turnaround for fabrication of a wide variety of designs and geometries. In the current protocol, a fused deposition modeling system is used with acrylonitrile butadiene styrene (ABS) as the build material, and the printed part serves as a sacrificial mold for the subsequent phantom casting. Our experience has shown that ABS is well-suited for such use, since it is soluble in common solvents (e.g., acetone), and it has sufficient strength and rigidity to maintain mold integrity after removal of the support material (e.g., to prevent deformation or fracture of diminutive mold features). In the current protocol, mold integrity is further ensured through the use of solid printed models, although this comes at the expense of increased dissolution time. Use of hollow models may also possible in some cases, to enhance solvent access, and thus, reduce dissolution time. However, careful consideration should be given to the effect this may have on mold integrity. Finally, while the phantoms fabricated herein are based upon idealized representations of neurovascular structures generated using a common CAD software package, the protocol is expected to be amenable to the fabrication of more complex, patient-specific geometries as well (e.g., via use of model files generated by conversion of clinical imaging data into the .STL file format used by most 3D printers). Further details regarding the phantom fabrication process are provided in ~~Protocol~~ Section ~~s 1 and~~ 2 of the Protocol.

The second element of the protocol involves the use of an open-source plug-in for ImageJ to conduct the cross-correlation analyses[9]. This is coupled with the implementation of a simple statistical thresholding scheme (i.e. intensity capping)[10] to improve image signal prior to cross-

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correlation, ~~and~~ as well as a post-correlation vector validation scheme, the normalized median (NMT), to eliminate spurious vectors through comparison of each to its nearest neighbors[11]. Collectively, this allows imaging to be accomplished using equipment commonly found in many bioengineering laboratories ~~(e.g., fluorescence microscope with filtered, continuous white light)~~, thus eliminating the need for acquisition of many of the costly components of typical PIV systems (e.g., pulsed laser, synchronizer, cylindrical optics, and proprietary software). Further details regarding the video collection, image processing, and data analysis are provided in Sections 5 and 6 of the Protocol.

Figure 1 illustrates ~~the various components of~~ the PIV setup used in this ~~study~~protocol, which relies upon a fluorescence microscope equipped with a high-speed camera for imaging, as well as an external, continuous white-light source (i.e., metal halide lamp) for through-objective volumetric illumination. A ~~, which also includes a~~ variable-speed gear pump is used to ~~to enable~~impose recirculating flow of a transparent mock blood solution ~~of a transparent mock blood solution~~ through ~~PDMS-based~~the neurovascular tissue phantoms. The solution ~~–~~is composed of a 60:40 mixture of DI water and glycerol, which is a common substitute for blood in hemodynamics studies[12–14], due to: a) its similar density and viscosity, i.e. 1080 kg/m^3 & 3.5 cP, vs. 1050 kg/m^3 & 3.0 – 5.0 cP for blood[15, 16]; b) its transparency in the visible range; c) its similar refractive index as PDMS (1.38 vs. 1.42 for PDMS)[17–20] ~~20, 21~~, which minimizes optical and d) the ease with which non-Newtonian behavior can be introduced, if needed, via the addition of xanthane[21]. Finally, fluorescent polystyrene beads are used as tracer particles (10.3 µm diameter; 480 nm/501 nm excitation/emission). While neutrally-buoyant beads are desired, sourcing tracer particles with optimal fluid mechanical properties (e.g., density, size, composition) and emission wavelength can prove challenging. For example, the beads used herein are slightly less dense than the glycerol solution (1050 kg/m^3 vs. 1080 kg/m^3). However, the hydrodynamic effects thereof are negligible, given that the duration of a typical experiment is far shorter than the time scale associated with buoyancy effects (i.e., 5 min & 20 min, respectively). Further details regarding the mock blood solution formulation and ~~,~~ *in vitro* circulatory system setup~~video collection, image processing, and data analysis~~ are provided in Sections 3 and 4 of the Protocol ~~Sections 3 to 5~~.

PROTOCOL:

1. ABS-BASED SACRIFICIAL MOLD FABRICATION
    1.1. Design an inverse model of the desired tissue phantom using CAD software.
    ~~1.~~1.2. Print the model using a 3D printer with ABS as the build material.
    ~~Design inverse model of desired tissue phantom using CAD software. The design used in~~
    2.1. Mixing
        2.1.1. ~~In a disposable weigh boat,~~ Mix the PDMS prepolymer base and curing agent in a mixture provides sufficient material for fabrication of phantoms with volumes up to 50~~XXX mc~~cm^3 ~~of 60g base to 6g curing agent for 5 to 10 min~~.
    ~~–~~Place the mixture in a vacuum desiccator for 60 min to degas and minimize bubble ~~–~~entrapment. Use cyclic pressurization/depressurization to facilitate bubble
    ~~–~~Casting
        2.2.1. Mount the printed ABS mold on a glass slide using molding putty to seal the

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interfaceMount printed ABS mold on a 75mm x 25mm glass slide such so that the top

Carefully pour the PDMS mixture into the mold while trying to minimize bubble entrapment. Lingering bubbles can be manually ruptured using a needlePour PDMS

2.2. Curing

temperatures this process can be accelerated[22].

2.3. Demolding

2.3.1. Dissolve the ABS by submerging the phantom in acetone and sonicating for at least 15 min using powers up to 70 W.

Place in ultrasonicaultrasonic bath to facilitate the dissolving of the ABS.

2.3.2. Thoroughly rinse the phantom with isopropyl alcohol, and then DI water to remove solvent residuesRinse phantom with isopropyl alcohol and DI water and check to

phantom is rinsed and dried sufficiently[23].

o

2.4. Phantom fidelity verification

2.4.1. Using an optical microscope with attached camera and image capture software, capture an image of a critical feature within the phantom under a magnification that maximizes the feature within the field of view.

Save the imagean image of the phantom under magnification. (For the example data

2.4.3. Load both images into ImageJ by dragging them onto the "ImageJ Ttoolbar".

2.4.4. MakeClick on -the calibration reticle image to make it active, and then select the "Lline" tool. Using the mouse, d-Draw a line along a feature of a known distance and select "Analyze" > "Set Scale…" from the ImageJ menu.

2.4.5. In the "Sset Sscale" window, the field labeled "Distance in pixels" should be pre-populated with the length of the drawn line in units of pixels. Enter the length of the feature on the calibration window in the field labeled "Known Ddistance", and its units in the field labeled "Unit of Llength". Check the box labeled "Global" to apply this calibration factor to all open images.

2.4.6. Make the image of the phantom critical feature active and use the "Lline" tool to draw a line along a feature of interest.

2.4.7. From the ImageJ menu, select "Analyze" > "Measure" (or press Ctrl + M) to measure the length of the line.

Compare the expected value against the value in the column marked "Llength" in the the "Results" window to confirm phantom fidelity.

3. MOCK BLOOD SOLUTION FORMULATION

3.1. Mix DI water and glycerol in a 60:40 ratio (by volume). A 100 mL volume is sufficient for the *in vitro* circulatory system described hereinMix 100 mL of 60:40 solution of DI water

3.1.

mock blood solution.

3.2.3.3. n and Hhomogenize the mixture on a magnetic stir plateplate at 400 rpm for 10 min.

10.3 µm diameter beads with 480 nm/501 nm excitation/emission wavelengths

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4.1. Pump Setup

4.1.1. Use a wire stripper tool to cut off the DC-end plug from the A~~/~~C to DC adapter source.

4.1.2. Strip the coating off the power and ground wires and connect them to the input terminal of the pulse width modulation (PWM) voltage regulator.

4.1.3. Connect the power and ground wires from the pump's DC motor to the output terminal of the PWM voltage regulator. The PWM's 7-segment -display outputs the duty cycle (0% - 100%) used to achieve a variable voltage to the DC motor.

4.2. Pump Calibration

4.2.1. ~~In a 500mL beaker,~~P~~.~~P~~p~~repare ~~1~~200 mL of mock blood solution (see S~~as outlined in~~

4.2.2. Place tubing from the pump inlet to the beaker holding the mock blood solution.

4.2.3. Place tubing from the pump outlet to an empty ~~500 mL~~ beaker~~.r. from the volume.~~

4.2.4. Select a desired ~~range of duty cycle values to~~ duty cycle set-point (0% - 100%). ~~and~~ timer. ~~and collect~~

4.2.5. Stop the timer once the pump has transferred ~~all~~the entire volume of ~~of the~~ mock solution. Use this time to calculate the volumetric flow rate.

~~-~~4.2.6. Repeat steps 4.2.1 – 4.2.5 for at least 5 different ~~varying flow rates~~duty cycle set-a least-squares regression curve. A minimum of three replicate points per duty cycle set-point is recommended. This relationship can be used to correlate the desired flow rate to the required PWM duty cycle.

4.5. VIDEO COLLECTION

~~-~~Image calibration

~~-~~Determine calibration ratio for the video imaging (see Section 2).~~Within the PCC~~

~~- Import this image into ImageJ to estimate the calibration constant (e.g., pixels/mm).~~

~~-~~Place the PDMS phantom on the stage of the ~~upright~~ fluorescence microscope.

5.2.2. Connect the phantom to the~~a~~ gear pump and introduce the mock blood solution.

~~o~~

with mock blood solution~~.~~. ~~, which~~ This may be particularly beneficial for models with vessels and/or blind features ~~(e.g., small perforators and aneurysm sacs, respectively)~~.

~~o~~

5.2.3. Set the pump motor controller for the desired flow rate based on the pump calibration curve.

~~-~~Run the pump for 1 ~~to~~ 5 min~~utes~~ prior to the experiment to ensure steady-state conditions.

~~-~~Turn on the ~~Mg-Halide~~external lamp to ~~i~~illuminate the field of view ~~of the camera according~~ based upon the ~~to the~~ excitation wavelength of the fluorescent beads.

~~-~~Adjust the imaging focal plane to the vessel mid-plane. This can be achieved by ~~:~~

~~:~~

phantoms with circular vessel cross sections); and/or

feature designed to facilitate identification of the vessel mid-plane~~. In the example~~

~~4.1.~~ Video recording

~~-~~Select the video recording parameters to optimize signal-to-noise ratio (SNR). parameters include exposure time, frame rate, and gain.

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frame rate of 2000 ~~frames~~fps~~/s~~, and a gain of 1.0. However, these parameters may on application (see~~; refer to~~ D~~d~~iscussion section for ~~more~~further details).

~~○1.1.1. Gain can be used to amplify the signal, but this also increases sensor noise.~~

~~-~~ Phantom clean up

~~-~~ If bead-sticking is observed after an experiment, sonicate the phantom in an aqueous aqueous ~~Alconox~~ detergent solution using powers up to 70 W.

~~5.~~6. IMAGE PROCESSING AND DATA ANALYSIS

~~-~~ Image pre-~~filtering~~processing~~processing~~

~~○~~ Drag the saved AVI file onto the I~~i~~mageJ window to ~~i~~import it. ~~AVI file into ImageJ in~~ "C~~c~~onvert to G~~g~~rayscale".

~~-~~ From the "ImageJ" m~~M~~enu, ~~S~~select "Analyze" > "Generate Histogram" (or press ~~c~~Ctrl H) to ~~Intensity capping~~ standard deviation for the unprocessed image.

NOTE: At high frame rates, it is not unusual for the distribution to be heavily toward zero (i.e., no signal).

6.1.3. From the "ImageJ" ~~ImageJ~~ m~~M~~enu, ~~S~~select "Image" > "Adjust"> "Brightness and press Shift + Ctrl + H) to ~~A~~apply a brightness/contrast filter.

6.1.4. On the "B~~b~~rightness and C~~c~~ontrast" menu, ~~P~~press the "Set" button to define the limits. ~~Choose~~Set the minimum value to be ~~such that the intensity limits are set by set~~ the maximum value to be the maximum intensity of the image (both based on statistics obtained in Section ~~5.1~~6.1.2).

~~○~~ NOTE: ~~obtained in step 5.1.2.~~ This typically eliminates all but the top 10% of standard deviations may be varied depending on the desired distribution of pixel intensities.
operation is provided in the Supplemental Material.

~~-~~ From the "ImageJ" ~~ImageJ~~ m~~M~~enu, ~~S~~select "Process" > "Noise" > "Despeckle" ~~-~~ number of saturated pixels.

NOTE: This operation is necessitated by the increased potential for pixel saturation that arises during optimization of brightness and contrast, which can produce spurious vectors during subsequent cross-correlation.

~~-~~ From the "ImageJ" ~~ImageJ~~ m~~M~~enu, ~~S~~select "Process" > "Filters" > "~~-~~Gaussian Blur" radius of 1.5 to reduce artifacts arising from the ~~. Select the radius to be 1.5. This value~~ pixels in a 3x3 neighborhood by the prior despeckling operation.

~~○~~ Click on the "P~~p~~olygon" tool, and then click within the image to outline the ~~The~~ interest (ROI).

~~-~~ From the "ImageJ" ~~ImageJ~~ m~~M~~enu, select "Edit" > "Clear Outside" ~~definition~~to locations where no signal is expected (e.g., areas beyond vessel wall boundary), which can decrease overall SNR.

~~o The ImageJ *ROI Definition* function, followed by the *Clear Outside* function, can~~

NOTE: This portion of the protocol ~~e section below~~ employs a third-party PIV plug-in for ImageJ~~ for conducting PIV analyses, . Ensure the plug-in has been installed before implementing any of the operations below.~~which relies upon Gaussian peak-fitting to enable estimation of displacement with sub-pixel accuracy.

6.2.1. From the "ImageJ" ~~ImageJ~~ m~~M~~enu, ~~Cross correlation~~Sselect "Plugins" > "Macros" saved macro "Supplemental Cod~~d~~e 2.ijjm" to ~~perform a batched correlation on~~cross-

NOTE: The ~~code performs an algorithm~~macro ~~is~~proceeds as follows:
within consecutive images is first performed to determine the local displacement of advected tracer particles, i.e. ~~(i.e.~~ the first image pair ~~is~~consists of the first and images~~images #1 and #2~~, ~~and~~ the second image pair ~~pair is~~consists of the second two-step multi-pass evaluation ~~was adopted~~is then performed with initial and interrogation window sizes of 256 x 256 pixels and 128 x 128 pixels, respectively; and finally, 3) ~~. After the correlation is performed for all image pairs,~~ the macro ~~potential for introduction~~appearance of spurious vectors.

~~o~~

6.3. Normalized median test (NMT)

6.3.1. From the "ImageJ" ~~ImageJ~~ m~~M~~enu, select "Plugins" > "Macros" > "Run…" and saved macro "Supplemental Code 3.ijjm" to ~~perform a batched vector validation~~ normalized median test.

~~NOTE: The macro algorithm performsis~~proceeds as follows: 1) ~~Perform~~ field is first compared to its eight nearest neighbors ~~and~~to compute the median 2) ~~.~~
neighboring vector and the ~~median vector magnitude~~calculated median; ~~3)~~ t
vector under investigation and the median neighboring vector value is then normalized by the median of the residuals; 4) ~~.~~
value (typically 0.2 pixels), which can be varied based on *a priori* knowledge of noise during image acquisition; and finally, 5) ~~.~~
instantaneous vector fields ~~before averaging them, since~~ is performed to produce a composite field, as this has been shown to increase the vector field quality[24].

~~o~~

REPRESENTATIVE RESULTS:

Figure 2 illustrates the PDMS tissue phantom fabrication process. The phantoms designed herein are intended for the study of flow in idealized wide-necked, saccular, intracranial aneurysms, as well as proximal branching perforator arteries. Important additional design features include: 1) a common reservoir that all vessels drain into, to ensure unencumbered fluid egress from the phantom, otherwise droplet formation may occur at the smaller vessel outlets; 2) a bubble trap, to facilitate bubble removal; 3) an outer cavity wall, to ensure parallelism of the vessel with the horizontal plane, as well as precise definition of the final phantom slab height, length and width; and 4) use of a 21 gauge hypodermic needle shank (820 µm nominal outer diameter) for molding

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of the perforator artery, due to our printer's inability to define such features with sufficient fidelity. Faithful reproduction of all design features is observed throughout.

Representative results for PIV-based flow characterization performed using the current protocol are presented in Figures 3 and 4. These studies were performed using phantom inlet flow rates of 100 mL/min, data acquisition rates of 2000 fps, and temporal averaging over spans of 0.05 s. Figure 3 shows representative image frames within the perforator artery, before and after intensity capping as well as corresponding surface plots of the 8-bit pixel intensity values. Both demonstrate that intensity capping significantly increases peak definition above the noise floor (i.e., increases SNR), which is critical to ensuring accuracy when performing subsequent cross-correlation. Figure 4 shows the effects of intensity capping and NMT operations on the velocity vector field. Marked improvement in field uniformity is observed, thus further underscoring the importance of maximizing SNR to minimize data dropout.

FIGURE AND TABLE LEGENDS:

Figure 2: PDMS-based tissue phantom fabrication process: a) CAD model of neurovascular phantom mold; b) Printed ABS mold, after removal of support material; c) Casting and curing of PDMS within the ABS mold; d) Partial dissolution of ABS mold material; and e) Completed PDMS phantom, with inset showing final dimensions of ~~key~~ critical features, as well as the region of interest (ROI) in the perforator artery where the PIV measurements were made. ~~.~~

Figure 3: Effect of intensity capping operation on image SNR. Representative image frame and corresponding pixel intensity surface plots within the perforator artery, before (a, b) and after applying the intensity capping operation (c, d).

Figure 4: Effects of intensity capping and NMT operations on velocity vector fields: Representative instantaneous velocity vector field within the perforator artery derived from: ~~(~~a) unprocessed image data; ~~(~~b) intensity capped data; and ~~(~~c) intensity capped data + NMT post-processing.

Figure 5: Effect of interrogation window sizing on correlation quality. Optimal window sizing occurs when the value of the zero-normalized correlation coefficient is maximized, and the standard deviation is minimized.

DISCUSSION:

The protocol described herein outlines a simplifie~~d~~ ~~and relatively low-cost~~ method for performing PIV studies to visualize neurovascular flows at physiologically-relevant dimensions and flow conditions ~~*in vitro*~~*in vitro*. In doing so, it serves to complement protocols reported by others that have also focused on simplifying the quantification of ~~ying~~ vector fields, but within very different contexts that require consideration of far larger length-scales[25~~23~~] or lower flow rates[26, 27](e.g. atmospheric or microcirculatory flows), and thus, reliance upon ~~simplification~~

schemes that are incompatible with the current application.

~~o~~ The most important considerations for successful implementation of PIV lie in the of flow field artifacts and maximization of image quality. Several steps in the tissue phantom fabrication process are critical to both of these criteria. For example, thorough degassing is crucial since air entrained within the PDMS during mixing can lead to bubble formation within the final phantom, which can adversely affect both feature fidelity and optical clarity. Additionally, minimization of surface roughness of the ABS mold is desired, since the PDMS casting process faithfully reproduces even the most minute imperfections (e.g., build lines, surface pores, scratches, etc.), thus resulting in surface roughness in the final phantom that can decrease optical clarity and increase potential for bead accumulation. While the protocol described herein has proven sufficient for the current application~~beyond the scope of the current protocol, ,~~ there are means for reducing such roughness, should there be need (e.g., acetone vapor smoothing[28], or optimization of layer thickness and part orientation with respect to build direction)[29]~~)~~.

$$f_{sampling} > \frac{v_{max} \cdot c_{calibration}}{h_{interrogation\ window}} \quad (1)$$

where $f_{sampling}$ is the camera acquisition rate (Hz), v_{max} is the maximum expected velocity (mm/s), $c_{calibration}$ is the calibration constant (pixels/mm), and $h_{interrogation\ window}$ is the size of the interrogation window (pixels).

correlation quality estimation techniques, such as the zero-normalized correlation coefficient[11]. In this technique, the averages of complementary signals from each frame-pair are first subtracted, and then normalized by the standard deviation of their intensities[11]. If a displacement of the original signal exists, such that all peaks and valleys match, the time-shifted value of this signal will be equal to one. Conversely, if there is no displacement that can align these signals, the value will be zero. This information is included in the ImageJ PIV output for each vector, and it can be plotted as its own field to ~~see if~~verify whether there are spatial effects contributing to correlation (e.g., uneven lighting). The correlation coefficient can also be averaged over a field as an overall estimate of its quality. Finally, this quantity may also be plotted against varying frame rates or interrogation window sizes to determine an optimum. Figure 5 illustrates the results from such an analysis using a Monte-Carlo synthesized particle field with displacements consistent with our experimentally-measured flows (a typical technique for characterizing correlation quality[11]). The results show that the interrogation window size and frame rate should be chosen such that a particle field is displaced by ≤ 20% of the interrogation window size per frame-pair to maximize the correlation coefficient, while minimizing its variability.

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DISCLOSURES:

The authors declare no competing financial interests.

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