JoVE Manuscript Changes: Responses to Comments by Reviewer 1.

**Manuscript title:** “Methods for Measuring the Orientation and Rotation Rate of 3D-Printed Particles in Turbulence”.

Formatting note:

* Reviewer comment.
  + Response.

**Edits made in response to comments from the reviewer:**

* Although the authors present another key concept of 3D printing as a central one, it seems to be not necessary to specify which method the users might find useful to create their particles: molding, printing, micro-machining or lithography, to name a few.
  + We agree that many different fabrication methods are possible. JoVE wants authors to focus on the specific methods used in a specific experiment and that is why the paper is written as it is.
* Another confusion that comes from the abstract is “Methods for producing on the order of 10,000 fluorescently dyed particles are described.” It is not clear why we need the methods also for fluorescent dyed particles and even more unclear is the 10,000. What if we just measure the motion of 1 or 100 particles or we need 100,000 particles?
  + Added a few lines to the Discussion to address why relatively low particle concentrations were used in these experiments. This paragraph, which is about the utility of the image compression systems, also addresses why it is beneficial in this apparatus to have on the order of 10,000 particles instead of just 1 or 100. We agree with the referee that a more general style is standard in our field, but JOVE wants precision about the experiments done.
* It continues with “four simultaneous video images” - what does it mean? four video cameras are involved? are these synchronized?
  + Clarified the phrasing in the abstract by changing “four simultaneous video images” to “four synchronized videos”.
* how tight is the synchronization requirement?
  + External trigger on cameras provides synchronization at the level of a few microseconds and is much more than enough for our purposes here.
* Although the title claims that the flow is “turbulent”, the abstract says “low-Reynolds” - does it mean that we need to adjust the title to the low-Reynolds turbulent flows?
  + The Reynolds number is too low to have an inertial range in the Eulerian structure functions, but is still high enough that the flow is fully turbulent.
* I would strongly suggest to improve the abstract’s clarity.
  + The abstract’s phrasing and terminology have been improved.
* Another general statement is the specificity of the experiment presented - the flow is quite synthetic (grids, specific size, oscillating in phase, homogeneity, etc.) and not clear why the users cannot use different camera arrangements, different image software, different tracking software? After all, the paper is well prepared, the method is explained thoroughly and could be definitely made less explicit and applicable for more general cases. If it’s understood correctly, the general statement suggests that if we’d use a rod-type balloons connected together in a jack form, we could measure rotation of a hot gas in a ventilation system.
  + See response to the comment about step 1.1.1 of the Protocol, below.
* 9. Is there any additional information that would be useful to include? yes, the software code of calibration, tracking, rotation assignment, Euler angles and solid body rotation rates. if compression is necessary, then it is also required for the users to be able to replicate the experiment.
  + Many of these will indeed be included with the final manuscript.
* 11. Are any important references missing and are the included references useful? yes, some references are missing and some are corrupted. the suggestions are given below
  + References have been fixed.
* abstract: “any shape that can be modeled on a computer using multiple slender rods” I’m not sure that it really means “any shape”. It is more likely that some shapes, altough can be modeled by slender rods will not doable, if, for instance, the rods will start obscure each other from the cameras (a sort of a optical density measure).
  + This is true, and shadowing due to arms is addressed in the Discussion. We largely rewrote this paragraph.
* p2 l70 “and the rotational motions of these particles are identical to those of their respective effective ellipsoids” - without going deeper into literature I would claim that this is only partially true - the effective force and moment on a particle is a surface integral over a solid surface of a particle and not an integral over an “equivalent” volume of a fluid which might also have some flux out of the volume of an “effective ellipsoid”. Whether the behavior is identical or not, remains to be studied in more details in each separate case, definitely in turbulent flows. It is suggested to add a clear statement about the degree of applicability that is not known *a priori* and shall be studied carefully by the users of this method. It relates to the following statement “Particles with symmetric arms of equal length rotate like spheres.” - in some sense the authors mean that the symmetric arms experience a symmetric forces and moments, similarly to the sphere, but definitely not the same ones. It could be that the authors mean that the size of the particle is also a parameter and maybe for very small particles (the size of which I could not deduce right now) this statement is more correct, than for large particles. Although the simulation of spheres compared to the rotation rates of jacks and crosses show indeed the similarity, it does not mean at all that instantaneously these bodies would rotate similarly. Statistically, they do experience a similar rotation rate, but that is a *completely different statement*.
  + This is covered in reference [2], where it is shown that small axisymmetric particles composed of slender arms have dynamics in turbulence equivalent to those of corresponding ellipsoids, called “corresponding effective ellipsoids” in the manuscript. It is indeed rigorous, but only true for small particles for which the velocity gradient is uniform across the particle. For larger particles that the referee seems to be referring to, the rotations indeed depend on shape in much more complicated ways.
* p2 l84 “ Extension of these methods to tracking rods was done by Parsa et al [5]” - I have looked for some information in ref. [5] (the journal is not given, but a quick Google search reveals it’s also in PRL, 2012) and ref. [6], but could not find any relevant information regarding of this statement “extension was done”. The references do not explain what extension was done or how it is done, except that the rotation rates are fitted with quadratic formula. There are two things to correct here: a) provide actual information on how the tracking methods of refs [3,4] are extended in order to provide the rotation rate measurements. b) why quadratic formula is used and how this affects the results.
  + Apologies about the incomplete reference. Issues related to references have been fixed.
  + This paragraph has been edited to clarify the relation of Parsa, *et al* to previous experiments and to the measurement methods presented here. Parsa, *et al* used stereoscopic images from multiple cameras to track the position and orientation of rods in turbulence, and the quadratic formula mentioned in that publication is simply the way the data was smoothed. It is unrelated to the measurements and methods discussed in this manuscript.
* Protocol: 1.1.1. why the slender diameter is 0.3 mm and why the length is 3 mm?
  + Earlier versions of this manuscript provided more leeway within the experimental procedure, but the editors wanted it to be more definitive and confined to our specific experiments. The procedure here details how these specific experiments were done, but we acknowledge that similar results could have been found with many types of turbulent flows, particles, and experimental apparatus.
* 1.1.6 - why is it 3D printer involved? and not some other production method? I’d say it’s redundant in a view of suggestion 1.2 - order ...
  + See previous response.
* 1.2.1 this is very important to understand what is the reason for “Ensure that particles are printed on high-resolution mode.” It is important for the users to know what does the “high resolution” means from the practical point of view of the experiment? the surface roughness? the length of the rods? the diameter? the connectivity in the center? the symmetry?
  + Clarified with the addition of the following: “[print on high-resolution mode] because the particles are near the minimum feature size of many 3D printers and the arms will not be as symmetric and may break if printed at lower resolution.”
* 2.1 - probably instead of “Prepare water in which the particles are neutrally buoyant.” - prepare a liquid in which the particles are neutrally buoyant.
  + Changed “water” to “a salt solution”.
* or maybe even prepare an experiment - why the issue of measuring rotation rates has to deal with the question of buoyancy? if the users would like to implement the method in air and prepare the particles inflatable with helium? or use them in a positively or negatively buoyant conditions?
  + We have studied the neutrally buoyant case. There is a lot of interesting physics in non-neutrally buoyant particles, and there are many ways to adjust particle buoyancy. At this point in the process, we just need neutrally buoyant particles for processing.
  + We added “to minimize particles’ arms bending while in storage and so that gravitational and buoyancy forces do not have to be accounted for in the analysis”.
  + See also response to the comment about step 1.1.1.
* 2.1.1.2 “different water densities” - probably “different solution densities”
  + Changed “water” to “solution”.
* 2.1.3 - not clear at all how the authors know how much salt (and of what type, maybe one would use magnesium salts?) to mix with what solvent (why water?) to get the density of the particles?
  + This is found through a calculation involving the density of the water and of the CaCl2 that we did not include in the manuscript.
* Why not to print the hollow particles that might even float in water?
  + We are interested in the neutrally buoyant case for the reasons included in the addition to step 2.1: “so that gravitational and buoyancy forces do not have to be accounted for in the analysis”.
  + Hollow particles could have densities that change with time as water diffuses in, making analysis and interpretation of results more difficult. See the second response to the comment about the note after step 2.2.4.
* in 2.1.4 the temperature is mentioned for the first time - I’d suspect the temperature has a lot of significance in all the discussed experiment’.
  + Storage temperature, within reasonable limits, does not have much of an effect in these experiments. This detail, “at room temperature”, was included because in an earlier draft of the manuscript we were asked to include the temperature at which the particles are stored.
* 2.2. this item is completely not clear. what is the resin and what are the small sections and…
  + Changed the beginning of this step to say, “Manually loosen the support material in which the particles come encased by gently…”.
* what is “manually massage”
  + This will be shown in the video.
* wouldn’t the NaOH solution remove all the resin without this preparation step? is it only for a specific printing material?
  + Clarified why manually removing some of the resin is done: “Remove excess resin in this way to reduce the amount of the NaOH solution that will need to be created for steps 2.2.1 – 2.2.4.”
* this is a very interesting note: “If particles are not stored in a density-matched solution, some arms may bend. Keeping them immersed in the density-matched solution for several hours also allows some voids in the plastic to fill with fluid.” first the bending - how the user would verify that the arms are not bend somewhere along the handling process? and how does one assure that the rotation rates later on are not measured for a biased subset of particles?
  + We have looked carefully at random samplings of a large number of our particles under a microscope in order to check that storing them in a density-matched solution minimizes bending. Some arms, of course, do have small deviations due to the handling process and these particles end up giving worse orientation matches.
* Second, if there are cases where particles are used with voids - their buoyancy is different and their interaction with the fluid is different also (e.g. an air cushion around it? small bubbles released when particles move?) - have the authors noticed something of this kind?
  + We have noticed that particle densities change with time over the course of being stored in density-matched solutions and have interpreted this as the salt solution filling voids in the particles. Part of the reason that we store the particles before performing the experiments is to have as many of these voids filled as possible before experiments begin.
* what does it mean “Dye particles with Rhodamine-B mixed with water” ? if the liquid is not water anymore, but some saline solution - is it possible to dye the particles? is Rhodamine B adhesive to the particles that spent some time in the bath with NaOH? is the 0.5 g/L a normal concentration of the Rhodamine B for any kind of particles?
  + The 0.5 g/L concentration of Rhodamine-B was arrived at by testing different concentrations, as were the duration and temperature of the dyeing.
  + For more recent experiments with softer plastics, we have tried dyeing particles in Rhodamine-B mixed with a salt solution in which the particles were neutrally buoyant. The dye enters the particles just as well in a salt solution as in a water solution.
* The heating of the whole thing is also questionable in a view of the previous discussion of the bending of particles and density matching.
  + Clarified this by adding “using too high of a temperature will result in the arms bending.”
* 3.1.1 what are these requirements for? 1 megapixel and 450 fps? is it for any Reynolds number turbulent flow? Can the user use some other cameras for different purposes?
  + See response to the comment about step 1.1.1.
* it is not clear (as the rotation tracking is not described clearly) why “Using more than four cameras could likewise decrease orientation measurement uncertainty.” shouldn’t redundancy help in this case?
  + Added to the end of this step to clarify this point: “Using more than four cameras could likewise increase orientation measurement precision because it will reduce the chance of arms being shadowed on all cameras, which is a primary source of uncertainty.”
* 3.1.3 why “Minimize optical distortions by building viewing ports into the apparatus perpendicular to each camera viewing direction” this requirement is important? how the distortions affect the rotation measures?
  + In tracking small spherical tracers we have found small (~20 micron) stereomatching errors when we look obliquely through a window into the apparatus. For the rotation measurements we have only used perpendicular viewing, but we expect that oblique viewing would very slightly degrade orientation measurements.
* 3.1.4 - why 200 mm macro lenses? these are extremely expensive lenses? why other lenses wouldn’t work?
  + Added “to obtain the desired measurement volume from a working distance of half a meter” to clarify the reasoning behind choosing these lenses.
  + These are expensive lenses, however they are still much less expensive than the cameras.
  + See also the response to the comment about step 1.1.1.
* is the size of the volume limited only to 3 x 3 x 3 cm?
  + This is a reasonable compromise between the desire to have high-resolution images of the particles and to track them over long periods of time.
* 3.1.5 - how does the mask look like? is it specific for rotation measure?
  + The mask is simply a grid of points used to identify common points on each camera and thereby create a common 3D coordinate system for all four cameras. It is not specific for rotation measure.
  + The mask will be shown in the video.
* 3.1.5.3 what is this aperture ? #f11 ? why are the filters? what are these B+W 040M 4x filters? why the filters are positioned after the calibration?
  + Corrected the order of the procedure; filters should be positioned before the calibration is done. Thank you for pointing this out.
* why do we need those?
  + We need to filter out the green 532 nm light from the laser, which would otherwise obscure views of the particles.
* the references are incorrect on p. 6 l271 OpenPTV is ref. [4] and Ouellette is ref [3].
  + References have been fixed. Apologies for the difficulty.
* 3.2 - the use of the laser and fluorescent particles is not clear at all. the power of the laser has probably little effect as the light collected on the cameras is the fluorescence of Rhodamine? it’s also related to the later statement of triggering of the laser and the cameras at 450 Hz. Does it mean that the laser should be extremely powerful (Q-switched Nd:YAG with 50 W and
  + The laser power is spread out into a large area (the size of the viewing volume), so the fluorescence is not saturated by the incoming light. We have varied the laser intensity within our accessible range and find that the fluorescence intensity does not saturate in this range.
* also not clear why the users in this method are instructed to follow the homogeneous portion of some flow (and why ref. [8] is relevant?) what if the user measures rotation in a jet?
  + We have been interested in homogeneous flows. [8] discusses the homogeneity of this flow and the limits to the assumption of complete homogeneity. We also think these methods would be useful in inhomogeneous flows.
  + See also response to the comment about step 1.1.1.
* how item 4.1.1 relates to this method? what if the users do not have image compression system at all?
  + After reading the Discussion, the reviewer commented, “the image compression need is clear now (ignore the comments above)”.
* what if the users would use another type of tracking system? a commercial one? or another real time image compression types, for instance by Kreizer et al (Exp. Fluids 2010, 2011)? it is interesting to understand whether the same principles will work with the defocusing, or astigmatism tracking systems (Kahler, Cirpka, etc. Exp. Fluids 2012, 2014)
  + See the response to the comment about step 1.1.1. The methods detailed in this paper are not the unique ways to experimentally arrive at these results; they are the methods we used in these experiments.
* the note “Because each particle typically covers approximately 5,000 bright pixels” really confusing - 5000 pixels each particle? does it mean that in the volume one shall have 5 to 10 particles in a time?
  + This is partially explained later in that same sentence and in the Discussion: “there is rarely more than one particle in view at a time” (from the note) and “Particle concentrations in these experiments were about 5 x 10-3 cm-3, which meant that typically only about 20% of images from the cameras had a particle” (from the Discussion).
  + We use a small field of view in order to obtain a large number of pixels for each particle in order to obtain highly accurate orientation measurements.
* 4.2 - again, why the setup is so specific? why 1 x 1 x 1 m? why turbulent by two parallel 8 cm mesh grids? why in phase?
  + See response to the comment about step 1.1.1.
* recording items again have a lot of very specific details which will likely be irrelevant for most of the users.
  + See response to the comment about step 1.1.1.
* 5.1.3 - 5.1.3.1 are probably some key components that will help to deduce the orientation of the particle. this would be very useful to understand what are the “numerical models” - is it the light spreading/diffraction of the rods which are modeled?
  + Clarified this step by changing the sentence to “Using the camera calibration parameters from 3.1.5.6, project the two end points of each rod onto the cameras and then model the distribution of light intensity in two dimensions…”.
  + Also broke this up into two steps to help clarify.
* not clear from the description of 5.1.4-5.1.5 what is the orientation of the particle and whether the problem is ill-defined as the same particle can have symmetrical orientations that are all fitted?
  + This is the concern motivating step 5.1.6. We have also added a paragraph to the Discussion justifying this assumption.
* does it have any meaning for the following result if the rod positions are switched? i.e. if the particle is slightly not symmetrical and it’s a cross but of uneven length (an elliptical disk model) - how difficult it would be to orient the two axis of the ellipse?
  + This would be an interesting avenue to pursue but we so far have only used symmetric particles. This method could be used to track asymmetric particles, in which case this step would need careful attention.
* especially difficult seems to be the choice of 5.1.6 - are there any false results? any uncertainty with one the steps above?
  + Addressed this by adding a paragraph to the Discussion justifying the smoothing assumption in 5.1.6. Mean rotation rates are more than an order of magnitude below the threshold that would make false results an issue.
* p5 l453 again “Small jacks rotate just like spheres in fluid flows” shall has somewhere “statistically” or “on average” or “rotation rates distribute similarly to”
  + See the response to the comment about this in the Introduction. A small jack in a uniform velocity gradient rotates exactly like a sphere.
* p11 l490 “Traditionally, the fluid vorticity has been measured using complex, multi-sensor, hot- wire probe” - this is probably true only for “single point”. Otherwise, “traditionally” the same 3d tracking methods were used by the ETH Zurich group to show vorticity (and moments of vorticity) and longer before that by Dahm using scalar imaging, etc. Probably there are other methods that measured vorticity, like LDV, etc.
  + References to these groups have been added in the paragraph, but as the reviewer mentions it is very difficult to have a comprehensive review of the field, which is why ours is primarily focused on other methods of particle tracking.
* p11 l500 “3D-printed anisotropic particles can be small, down to 300 *µ*m in diameter” - this statement is somewhat different from the size given in the CAD program?
  + We think it is consistent throughout that particles have 3 mm arm length and 300 μm arm diameter. Also clarified the Discussion: “3D-printed anisotropic particles can be small, with arm thicknesses down to .3 mm in diameter”.
* p12 l525 … However, the shape of tetrads is not shown
  + They will be shown in the video. A screenshot of the tetrad was also included with the previous submission.
* and it’s also not clear whether the particles can be cubes or pyramids, i.e. not connected at the center?
  + These particles have been considered, but we decided not to pursue them because parts of these objects shadow one another more often. We expect that these methods could be used with a wide variety of particle shapes.
* Regarding the surface of the particle as one of the key parameters in adequate correspondence of the rotation to the real case of a spheroid, the question is how the results are similar/different if the jack/cross is embedded in a transparent ball? this can be a good verification of the general statement of “equivalence of rotation rates” and the processing method.
  + We agree that this is an interesting approach. The Bodenschatz group (MPI Gottingen) and the Variano group (Berkeley) have done experiments like this. We are quite confident in in the Stokes flow solutions that show that equivalent spheroids have identical rotations in uniform velocity gradients. (See Bretherton JFM 14:284 1962 and Marcus et al, NJP (Ref 1) for details).