**Editorial comments:**

The manuscript has been modified by the Science Editor to comply with the JoVE formatting standard. Please maintain the current formatting throughout the manuscript. The updated manuscript (55032\_R1\_070516.docx) is located in your Editorial Manager account. In the revised PDF submission, there is a hyperlink for downloading the .docx file. Please download the .docx file and use this updated version for any future revisions.

1. Formatting:

-Please use - rather than ~ to indicate a range (see 1.2).  
-1.4 – Figure 2 does not seem to be the right figure to cite here.

2. Grammar:  
-Line 47 – “in laminar stirred tank”  
-1.3 – “condition”  
-2.3 – “to linearly proportionally”  
-4.4 – “parameter”  
-Line 294 – “to large industrial vessel”  
  
3. Additional detail is required:  
-1.1 – What is the working fluid?  
-1.3 – How does one operate under laminar flow? Is this in a hood? Does the particular speed refer to the speed of the impeller? What speed is used here?  
-2.1 – How is the tank run at this speed (what part is set to 1100 rpm)?  
-2.2 – How is the camera oriented toward the tank (placement/distance, etc.)?  
-3.1 – Is the aperture typically opened or closed for this? What should one see when background is minimized? Are the lights turned off for this adjustment? Does the camera face the top of the liquid or the liquid from the side? This is unclear.  
-4.1 – How is the site of the ROI determined?  
-4.2 – How/where is the camera positioned?  
-4.3 – How many particles are used?  
-4.4 – How is calibration performed? What static target is used?  
  
4. Branding:  
-1.1 – Perspex  
-Figure 1 - Rushton  
  
5. Results:  
-Please discuss Figure 2 in the results section.  
-Figures 6 & 7 – Please explicitly describe each panel in the figure legends.  
  
6. Discussion: Please discuss the significance with respect to alternative methods (and include independent citations) as well as the critical steps of the method.

**Response:** All the editorial comments have been carefully addressed. All the typos have been fixed and the additional information have been given in the protocol. Additional discussion has been given in the discussion part to provide the comments on alternative methods.

**Reviewers' comments:**

**Reviewer #1:**

**General response:** We note that Referee 1 remarked that the paper “presents an interesting method for particle tracking and visualization in stirred tank system” and the technique presented will be “ very useful to validate computational models in the laminar and turbulent flow regimes”. We thank the referee for the useful comments and answer them in the following point by point.  
  
*Major Concerns:*  
There are various information missing from the paper. Here some suggestions:  
1. The position of impeller in the tank was not supplied. The aspect ratios affect the mixing process and flow regime. Moreover, the size of tank is smaller than impeller? (refer to table)

**Response:** The impeller was placed in the centre of the tank. The impeller size is 7cm in diameter, and the tank dimeter is 19cm.All the information have been given and/or corrected.  
2. What is the type of impeller?

**Response:** It is a 6-blade Rushton impeller. Information has been given in section 1.3 in the manuscript.

3. Compare results to studies performed in the literature to confirm data.

**Response:** We thank the reviewer for giving the valuable comments. Our next paper will provide more quantitative data, thus will allow us to compare results to available date in the literature.

4. In the protocol section: the discussion does not match the order presentation i.e. Figure 2 and 3 are in the wrong order.

**Response:** We agree with the reviewer that the story line and the figures do not match. We reordered the figures. We believe that the text is more clear.

5. Protocol 2.2: it says 20 mins but Figure shows 30 min as the final time.

**Response:** We thank the reviewer. The typo is corrected.

6. The coordinate system, (x,y,z) presented in Figure 1 does not match the figures presented in Figure 5.

**Response:** We agree with the reviewer that the coordinate system in Figure 1 is not identical to Figure 5. Figure 1 is replotted and the coordinate system is corrected now.

7. Figure 5(d) and (e) have not been discussed in the report - consider removing them?

**Response:** Figure 5d and 5e provides three-dimensional information on the flow. Hence, we believe that it is informative to show both figures. The text in the results section is rephrased to highlight the outcome form both figures.

8. Figure 7 - there is no comparison of flow patterns to results from literature  
**Response:** We thank the reviewer for giving the valuable comments. Our next paper will provide more quantitative data, thus will allow us to compare results to available date in the literature.

**Reviewer #2:**  
*Manuscript Summary:*  
**General response:** We note that Referee 2 remarked that the paper “would make an in interesting video presentation” and defined that “the instrumentation is well described” and the paper is suitable for JOVE. We thank the referee for the useful comments and answer them in the following point by point.

*Major Concerns:*  
1. Is the software open source?  
I think this paper will be useful provided that software for calibration and analysis of particle position is available, and its use explained in the video. Is some sample data and software going to be made available? I think that a reference to an open-source repository with this material would be very valuable.

**Response:** We thank the reviewer for the comment. As it is outlined in the manuscript, 3D-PTV is not a new technique, but a rather mature one and in this sense it has been validated over the last 20 years or so. The software is open source. What is completely new however, is its application in this novel trapping phenomenon.

2. Briefly mention sources of error in position estimation.  
How does motion blur of the particle images due to movement during the camera exposure time (or laser pulse duration) affect the precision of position estimation? If the laser pulse is ~10 ns, then this effect may be negligible, however in reference [6] which this paper seems to build upon, a 25 W continuous wave laser was reportedly used. In which case there may be significant blur during the camera exposure time (say, 1 m/s \* 0.1 ms = 100 microns). Is this an important source of uncertainty?  
Savin and Doyle (doi: 10.1529/biophysj.104.042457, Biophysical Journal 2005) have a good paper on position estimation (using 2D image data), and their "dynamic error" corresponds to motion blur. I wonder if this is a significant source of uncertainty in this work? Is this what leads to the 350 micron error in "3D space" position estimation?  
If not, then what are the sources of error in position estimation for the particles? Does error arise more from the alignment of the imaging system (view splitter and camera)?

**Response:** We thank the reviewer for the comment. The determination of the particle position from image to object space and the linking those positions in time, forms the main framework of 3D-PTV. On an image, an observed object occupies a patch consisting of a certain number of pixels. Therefore, the particle detection mainly relies on pixel width and grey scale intensity. The center of gravity of the particle is computed as the arithmetic mean of the pixel coordinates weighted by the associated grey values. This particle detection and determination of particle coordinates constitutes the first step of the PTV algorithm. Through a stereoscopic principle and careful calibration of camera positions and orientations, 3D particle positions are computed from 2D images of the particles observed from different viewing directions at a given time instant (Saha, Experimental Analysis of Aggregate Breakup in Flows Observed by Three Dimensional Particle Tracking Velocimetry, ETH Zurich, 2013).

Calibration, i.e. the determination of external and internal camera parameters, such as position, orientation, focal distance, optical axis correction, plays a significant role in obtaining an accurate result. To constitute the correspondences between images, it is crucial to obtain the particle position in real space with high precision. The calibration can be performed either statically or dynamically. The static calibration target comprises a number of points with exactly known coordinates. To acquire a robust calibration along the camera direction, the static calibration target is layered in different planes. The accuracy of the measurement is highly influenced by the calibration. The accuracy of the measurements in 2D is different from 3D as one expects to have relatively higher errors in camera direction compared to the other dimensions. In other words, velocities along the camera direction (z axis) are more difficult to measure due to the limited depth of field. Furthermore, optical aberrations, diffraction, lens distortion, inhomogeneous glass walls disturbing the incidence angle, local temperature gradients affecting the refractive index are the major causes that may reduce the image quality which potentially leads to the large errors in particle coordinate estimation.

3. Cartesian axis labels need to be checked (see Minor concerns - Figure captions).

**Response:** We agree with the reviewer that axis labels need to be rephrased. All figures are rechecked and now the axis labels are correct.  
  
*Minor Concerns:*  
The quality of the writing seems generally good, but I have a number of specific comments as follows.  
Specific comments  
Line 50. "tendency of" might mean "behavior of"  
Line 64. "match" should be "matches"  
Line 88. For equation 2, it should probably be stated that \nu is the kinematic viscosity (and not dynamic viscosity), since the other terms are defined.  
Line 107. "advent" might mean "advect"  
Line 122. "experience" might mean "expectation".  
Line 145. The reference should be to Figure 3 (in order of figures in my review copy.)  
Line 156. "attain" should probably be "obtain"  
Line 156. "pixel number distribution" is more usually written as "pixel value distribution" or "grey level histogram" in my experience.  
Line 172. What is the pulse duration of the laser (and its mean power or pulse energy)?  
Line 187. What does "image space" and "3D space" refer to exactly? Will the calibration be done using software based on reference [6]? If so, additional explanation will be needed (presumably this will be in the video).  
Line 201. The reference to Figure 3 should be Figure 2 (in my review copy) - switch around Figures 2 and 3 in the Figure list.  
Line 246. "turbin" should probably be "turbine"

Line 329. "limited" should be "too limited"

**Response:** We thank the reviewer and all the suggestions were implemented into the text.

Line 303. The particle separation is presumably only effective for particles of some particular size range. Is there space to discuss this?

**Response**: We thank the reviewer for the valuable comment. We agree that size effect is of interest of our research but for this study it is not in our scope. We will face our research to this direction in our future studies.

Caption  
Figure 1a. The Cartesian axes seem to be in a non-standard left-handed arrangement. This is inconsistent with Figure 5 which seems to use a standard right-handed arrangement. Please check the axis labels thoroughly. Please use a right-handed set of Cartesian axes if possible.  
Caption  
Figure 7b. Is the velocity map really for the XY plane (using the axes in Figure 1a), or is it the YZ plane (in the notation of Figure 1a)? Please check.

**Response**: We agree with the reviewer that the coordinate system in Figure 1 is not identical to Figure 5 and Figure 7. Figure 1 is replotted and the coordinate system is corrected now.  
  
*Additional Comments to Authors:*  
N/A  
  
**Reviewer #3:**  
*Manuscript Summary:*

**General response:** We thank the referee for the useful comments and answer them in the following point by point.  
The manuscript presents a method to study the movement of neutral density or lighter particles in a viscous Newtonian liquid. The manuscript presents actually three experimental techniques. First one involves a direct flow visualization which shows how particles introduced at the liquid surface during mixing are trapped in vortex rings created above and below the impeller blades. The second technique involves the introduction of LED capsules at the liquid surface at the beginning of mixing and track their trajectories using a high-speed camera placed above the liquid surface. This technique shows the presence of vortex rings above and below the impeller and the pathways traced by the capsules once they are trapped within the vortex rings thereby confirming the particle-trapping mechanism observed in the first technique. The third method employs a 3D PDV instrument to study the Lagrangian trajectories of particles under laser illumination. This technique provides not only the trajectories of particles but also the relative velocity vectors of the underlying liquid flow. Authors suggest that the phenomenon of particle clustering in the vortex ring can be used for solid-liquid separation in a high viscous liquid.  
  
*Major Concerns:*  
The title of the manuscript 'Visualization of particle focusing in chaotic flow' does not describe the method clearly. Especially the phrase 'focusing in chaotic flow' is not ambiguous,and needs to be rephrased. The solids used in this work are lighter than the liquid phase. Can the clustering technique be used for particles denser than the liquid phase? Can this technique be used in a continuous system where the feed is a slurry and the products are clear liquid and solid phases?

**Response:** We thank the valuable comment given by the reviewer. As in laminar flow tank, it has been quite well known that the flow is chaotic. We thus change our title to ‘Visualization of particle focusing in stirred chaotic flow. ‘We agree that particle-liquid separation is of interest of our research but for this study it is not in our scope. We will face our research to this direction in our future studies. However, we have had direct experimental evidence that heavy particles can be made to concentrate into the vortex tubes. This is very surprising, and we are doing more work with the colleagues from the states.

*Minor Concerns:*  
Page,  
Line no. Comments/Corrections  
59 The motion of particles is a common phenomenon encountered in nature and engineering. - Needs to specify the medium in which the motion of particles is common and important.

**Response:** We have added ‘ Examples include: fine sediment in water flow in the rivers, raindrops formed in the cloud, and tiny crystals produced in the multiphase crystallizer.’  
86 'where Vp is the velocity of a rigid spherical particle' - What velocity you are talking about? Is it the settling velocity or horizontal velocity of the particle?

**Response:** Vp is particle velocity relative to the surrounding fluids.

Fig 5 It will be useful if you could elaborate the caption by stating which is the side view, top view and front view.  
Fig 6 Same as above

**Response:** We have given more details in the captions.

145 Capsules with LED are shown in Figure 3 in the manuscript, not in Figure 2. Needs to change figures. What is the density of LED as compared to glycerine?

**Response:** we have modified the captions and orders. The density ratio is 0.71, and it has been stated in the manuscript.

150-151 Why did you choose 1100 rpm in this run? What is the Reynolds number? Is the flow laminar under this condition? What type of 'small' particles were used in this run?

**Response:** The working liquidglycerine has very high viscosity, and 1100rpm was chosen to maintain a laminar flow, with Re ~ 100. The particles are polystyrene, with diameter of 1.4 +/- 0.4 mm.

222 How was the particle residence time near the core of vortex ring was measured?

**Response:** Particle residence time corresponds to the time needed for a tracer particle to leave the region of interest. It can be calculated as;

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In our analysis, we define the region of interest as the region in the core of the vortex ring.

91 Change 'Whereas' to 'Although'.  
106 Change 'advent' to 'advect'  
154 'Take four images at t ~0 min, 1 min, 3 min, 5 min, and 20 min respectively'. The last sampling interval in the figure 3 is shown as 30 min, not 20 min. Which one is correct?

156 Change 'attain' to 'obtain'.  
161 Change 'Place the camera on the top of the liquid level' to 'Place the camera above the liquid level'.  
205 Change 'repeller' to 'repelling'.  
219 Change 'until it reaches to the vortex' to 'until it reaches the vortex'.  
236-237 Change '(Figure 7, left)' to '(Figure 7a)'.  
239-240 'the ROI and distinguish it from the two vortices whose cross sections are clearly visible in Figure 7.' - State in which of the Figure 7 (a or b)?  
246 Change 'turbin' to 'turbine'  
252 Change 'Reynolds speed' to 'Reynolds Number'  
299 Change 'attained' to 'obtained'

257 Is 'rolls' the correct the word to represent the vortex tube ? Can't you use 'rings' instead?

**Response:** We thank the reviewer. We have seriously considered the comments given by the reviewer, and have made all the necessary changes in the manuscript.