On the uncertainty of Astigmatic Particle Tracking Velocimetry in the depth direction

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Abstract Astigmatic Particle Tracking Velocimetry (APTV) is a single-camera 3D3C method able to track the three-dimensional displacement of tracer particles in a measurement volume. The measurement principle relies on an astigmatic optical system that provides aberrated particle images with a characteristic elliptical shape. The length of the principal axes of the particle images depends on the depth position of the corresponding tracer particles. This work studies the consequences that different optical arrangements and calibration approaches have on the final uncertainty of APTV. In particular, we used numerical Monte Carlo simulations to estimate the uncertainty of the different configurations. The simulations are based on an approximated analytical model of APTV recordings. An experimental test case was used to validate the numerical results. The results provide general guidelines to select the optimal optical arrangement and calibration approach for APTV experiments.

1. Introduction

The use of astigmatism as tool for measuring the position along the optical axis is a well-known concept and it is used for instance in CD/DVD players to determine the position between the disk and the laser head. A first application of this concept to locate the position of beads in a fluid was shown for instance by Kao and Verkman in 1994 [4]. This concept was further developed for 3D particle tracking velocimetry applications [1-3, 5]. In the past years, Astigmatic Particle Tracking Velocimetry (APTV) found its field of application mostly in microfluidics [6,7], where the use of multi-camera approaches is often difficult or not possible at all.

The optical arrangement of an APTV system is essentially composed by a primary stigmatic optics (e.g. a microscope, or a camera objective) and an astigmatic optics, typically a cylindrical lens placed before the camera sensor, as shown in Fig. 1.

Fig. 1 Schematic of the optical arrangement of an APTV system. A cylindrical lens in the optical path introduces an astigmatic aberration in the image. Particle images assume an elliptical shape.
As a consequence of the astigmatic optics, the global optical system does not have a single focal plane. In particular, we can identify two principal focal planes: one corresponding to the plane where the cylindrical lens is flat (xz-plane in Fig. 1) and the other corresponding to the maximum curvature of the cylindrical lens (yz-plane in Fig. 1). A particle image taken with this system can never be in-focus and it will show a characteristic elliptical shape that following the optical arrangement in Fig. 1 will have the principal axes oriented in the x- and y-direction of the image plane. Following the classical model of defocused particle images introduced by Olsen and Adrian [8] it is possible to give an approximated analytical description of the length of the principal axes of an astigmatic particle image as:

\[
\begin{align*}
 a_x(z) &= M_x \sqrt{c_1^2 (z - z_{fxz})^2 + c_2^2} \\
 a_y(z) &= M_y \sqrt{c_1^2 (z - z_{fyz})^2 + c_2^2}
\end{align*}
\]

(1)

where \( M_x \) and \( M_y \) are the magnifications in the x- and y-direction, \( z_{fxz} \) and \( z_{fyz} \) are the z-coordinate of the main focal planes, \( c_1 \) and \( c_2 \) are constants depending on the characteristics of the primary optics. More details about how these equations were derived can be found in [3, 8, 9]. It must be noted that Equation (1) is strictly valid only in the case that \( z \) is small compared with the distance of the particle from the objective lens (\( z << s_0 \)). In this case, \( a_x \) and \( a_y \) are functions only of the depth position of the tracer particles.

In a typical APTV measurement, the astigmatic image of a group of tracer particles suspended in the flow is recorded. After image processing, 4 variables are associated to each particle image: the in-plane positions \( X \) and \( Y \), and the axes length \( a_x \) and \( a_y \). A calibration procedure is then needed to retrieve the position of the particles in the physical space. The calibration in the x- and y-direction can be performed using conventional 2D-PTV approaches and will not be discussed here. The calibration in the z-direction consists in solving the following problem:

\[
z = f_c(a_x, a_y)
\]

(2)

where \( f_c \) represents a function or an algorithm. This problem is not straightforward since non-linear non-injective functions are involved and different approaches are possible. In this work we will consider four calibration approaches that have been proposed so far in APTV-related publications:

1. **diameter**: the \( z \) is related to the larger particle image diameter. The use of the larger diameter is needed to avoid ambiguities;
2. **difference**: the \( z \) is related to the difference between \( a_x \) and \( a_y \);
3. **ratio**: the \( z \) is related to the ratio between \( a_x \) and \( a_y \);
4. **euclidean**: this approach considers a parametric calibration curve in the \( a_xa_y \) space, with \( z \) as parameter. The point in the calibration curve closest to the measured pair \([a_x, a_y]\) identify the \( z \). More details about this method can be found in [3].

All the calibration approaches are in principle valid, however they will give different results and uncertainty figures in real systems as a consequence of the measurement error. In fact, an error in the particle image diameter determination will propagate differently according to the chosen calibration approach. The same will happen for the choice of the optical arrangement that will determine the shape of the calibration curves.

In particular, the magnification and NA of the objective lens, that determines the constants \( c_1 \) and \( c_2 \) in Equation (1), and the focal length of the cylindrical lens and its distance from the camera sensor, that determine the distance between the two focal planes:

\[
\Delta z = z_{fyz} - z_{fxz}
\]

(3)
Fig. 2 Different calibration approaches for APTV. The depth coordinate $z$ as function of (1) larger diameter, (2) diameter difference, (3) diameter ratio, (4) minimal distance from the calibration curve.

This work intends to focus on this problem and in particular to answer the following questions: how is the uncertainty affected by different optical arrangements and calibration approaches? Is there an optimal configuration to be used?

2. Materials and methods

An experimental test case was used as a reference for the numerical analysis. In particular, a typical APTV setup was used to measure the position of spherical polystyrene beads (Mikropart GmbH) with a 2-μm diameter. The APTV setup consisted in an inverted microscope (Axio Observer Z.1, Carl Zeiss AG) with a motorized focus-stage. The optics was composed by a LD Plan-Neofluar objective lens with $M = 20 \times$ and NA = 0.4 together with a cylindrical lens with focal length of 300 mm, placed at approximately 60 mm from the camera sensor. A 16-bit, 2560×2160 pixels, sCMOS camera (PCO GmbH) was used to capture the images. A water dispersion of the particles was let to dry off on a microscope slide that was then placed on the microscope stage. The particles were precisely displaced in different $z$-positions using the motorized stage of the microscope. A picture of the experimental setup is shown in Fig. 3.

The numerical analysis was performed by means of the Monte Carlo method. Specifically, the theoretical calibration curves corresponding to a given APTV setup were obtained from Equation (1). The calibration curves were used to obtain the respective $a_x$ and $a_y$ for a set of 6000 $z$-coordinates randomly distributed and afterwards an artificial error was added to each value of $a_x$ and $a_y$. The error was considered as normally distributed with a standard deviation calculated according to this model:

\[
\begin{align*}
\epsilon_{ax}(z) &= \epsilon_0 + k_xa_x(z) \\
\epsilon_{ay}(z) &= \epsilon_0 + k_ya_y(z)
\end{align*}
\]

The goodness of the model will be discussed in the result section. The simulations were performed using a custom made code in the Matlab environment. The built-in Matlab function `random` was used to generate the random data with the specified distributions.
3. Results

The experimental values of $a_x$ and $a_y$ as a function of $z$ are shown in Fig. 4.1. The functions in Equation (1) were fitted to the data and the following values were obtained: $M_x=22.2$, $M_y=18.2$, $\Delta z=-27.4 \, \mu m$, $c_x=0.15$ pixels/$\mu m$, $c_y=0.62$ pixels. The overall height of the measurement volume was $H=100 \, \mu m$, and the $z_{FLC}$ was set equal to 0. The results of the simulated measurement obtained with the numerical method are shown in Fig. 4.2. For the error model, the following parameter values were obtained from the experimental data: $\varepsilon_0=0.42$, $k=0.03$. The different calibration approaches were then used to estimate the $z$ from the $a_x$ and $a_y$. The comparison between experiments and simulations is shown in Fig. 5.

In Fig. 5.1 and 5.2 the measurement error $\varepsilon_z$ (calculated in terms of standard deviation of the measured $z$ from the true value and normalized over the measurement volume height, $H$) is plotted as a function of $z$. In general, the ratio and euclidean approaches are the ones providing the best performance. In particular, the ratio approach shows a good performance inside the two main focal planes, however the uncertainty increase significantly moving in the outer regions. Additionally, two peaks are located close to the main focal planes.

![Fig. 4 Particle image diameter $a_x$ and $a_y$ as a function of $z$ from experiments (1) and simulations (2).](image-url)
A similar behavior is also observed for the euclidean approach that shows however smaller uncertainty values. The diameter approach is directly related with the $\varepsilon_{ax}$ and $\varepsilon_{ay}$ and it provides however uncertainty values that are overall larger compared to the other approaches. The difference approach performs well between $z_{f_{ax}}$ and $z_{f_{ay}}$ but its uncertainty increases rapidly moving toward the outer regions.

The same considerations are in general valid for the bias error, reported in Fig. 5.3 and 5.4. In this case, the numerical simulations tend to underestimate the experimental bias error, especially with regards to the diameter approach. Overall, the results in Fig. 5 prove that the uncertainty figures and trends of the different calibration approaches are well predicted by the numerical simulations and are consistent with the experimental values. Afterwards, in order to check for the generality of the analysis, we tested the effect of different distributions of the particle image detection in the numerical simulations. Results are presented in Fig. 6.

Two cases were considered, one with the error proportional to the particle image diameters ($\varepsilon_{y}=0$) and one with constant error distribution ($k=0$). The scatter plots of $a_x$ and $a_y$ as a function of $z$ are shown in Fig. 6.1 and 6.2. For the case with error proportional to the particle image diameters, the results are basically identical to the ones in Fig. 5 as shown in Fig. 6.1. For the case with constant error distribution, the results are also consistent with the ones in Fig 5 except for the ratio approach that shows a significantly larger error. However, we can conclude that the distribution of the particle image detection error does not play a crucial role for the purposes of this study and in the following analysis we will use the values of $\varepsilon_{y}=0.42$ and $k=0.03$. Results obtained with this configuration are expected to be qualitatively valid for most applications except the ones where the error distribution is constant, which are however seldom encountered in real applications.

**Fig. 5** Error in the measurement of the $z$ coordinate (normalized over the height) for experiments (1) and simulations (2). Bias error as a function of $z$ for experiments (3) and simulations (4).
Finally, we tested the performance of the four calibration approaches on four different calibration curves (corresponding to as many optical arrangements). In particular, we consider two critical parameters of the optical arrangement: the degree of astigmatic aberration, expressed as the ratio \( \Delta z/H \) between the focal plane distance and measurement volume height, and the degree of defocusing (depending on the depth of field of the primary optics), expressed as the ratio \( \max(a_x)/\min(a_x) \). The calibration curves taken in consideration are plotted in Fig. 7 in the \( a_x,a_y \) space.

Fig. 7 Calibration curves used for the Monte Carlo simulation. (1) small astigmatic aberration \( \Delta z/H = 0.27 \), large defocusing \( \max(a_x)/\min(a_x)=15.4 \), (2) large astigmatic aberration \( \Delta z/H = 0.67 \), large defocusing \( \max(a_x)/\min(a_x)=20.2 \), (3) small astigmatic aberration \( \Delta z/H = 0.27 \), small defocusing \( \max(a_x)/\min(a_x)=3.3 \), (4) large astigmatic aberration \( \Delta z/H = 0.67 \), small defocusing \( \max(a_x)/\min(a_x)=4.3 \).
Fig. 8 Error in the measurement of the $z$ coordinate as a function of $z$ for the different optical arrangements reported in Fig.7. The data are normalized over the height $H$ of the measurement volume.

The results of the Monte Carlo simulations for the different configurations are reported in Fig. 8, where the $\varepsilon_z$ is plotted as a function of the coordinate $z$ (normalized with the measurement volume height).

The simulations show that in systems with a large defocusing effect (small depth of field compared to the measurement volume height), reported in Fig. 8.1 and 8.2, the *euclidean* and *ratio* approaches are the ones providing the lower uncertainty. In particular, the *euclidean* approach should be used with low or moderate astigmatic aberration whereas the ratio approach can be used with larger aberration. The *ratio* approach performs better between the two main focal planes, but gives high-localized uncertainty values in proximity of the focal planes. This aspect should be taken into account in the evaluation of the data.

The situation for systems with a small defocusing effect (large depth of field compared to the measurement volume height) is slightly different, and in this case the *euclidean* approach is the only one giving more reliable results. In particular, the *ratio* and *difference* approaches give similar uncertainty values between the two focal planes, but the uncertainty level rise significantly moving outside the central region of the measurement volume. Also, no significant advantage is observed in the central region in comparison with the *euclidean* approach. The *diameter* approach has the advantage to have a more uniform error distribution, however it always provides larger uncertainty values in comparison with the other approaches.

Finally, assuming the same error distribution in the detection of particle images, the simulations show that systems with larger defocusing effect provide a smaller overall error. Therefore, it is advisable in general to choose the primary optics as well as the size of the tracer particles in order to have a large value of $\max(a_i)/\min(a_i)$. However, too large particle images will enhance the occurrence of particle image overlap
that is problematic for the shape detection algorithms. This aspect should be also considered in planning an APTV experiment.

4. Conclusions

The effect of different optical configurations and calibration approaches for the APTV method has been investigated using numerical Monte Carlo simulations. A reference experiment was performed as well to provide a validation to the simulations. Four different calibration approaches have been presented and applied to four different optical arrangements. The simulations show that the euclidean and ratio approaches are the ones providing the lower uncertainty in systems with a large defocusing effect (small depth of field compared to the measurement volume height). In particular, the euclidean approach should be used with low or moderate astigmatism (expressed by the distance between the two focal planes Δz with respect to the measurement volume height H) whereas the ratio approach should be used for larger aberration (Δz/H ≥ 1).

In case of systems with small defocusing effect (large depth of field compared to the measurement volume height) the euclidean approach is the only one giving reliable results. Finally, assuming the same error distribution in the detection of particle images, the simulations show that systems with larger defocusing effect provide a smaller overall error.

References


