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High-speed particle detection and tracking in microfluidic devices using event-based sensing

Jessie Howell¹, Tansy Hammarton², Yoann Altmann^{3*}, Melanie Jimenez^{1*}

¹ Biomedical Engineering Division, James Watt School of Engineering, University of Glasgow, Glasgow, G12 8LT, UK

² Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow, G12 8TA, UK

³ School of Engineering and Physical Sciences, Heriot-Watt University, Edinburgh Campus, Edinburgh, EH14 4AS, UK

Corresponding author: Melanie.Jimenez@glasgow.ac.uk

Abstract

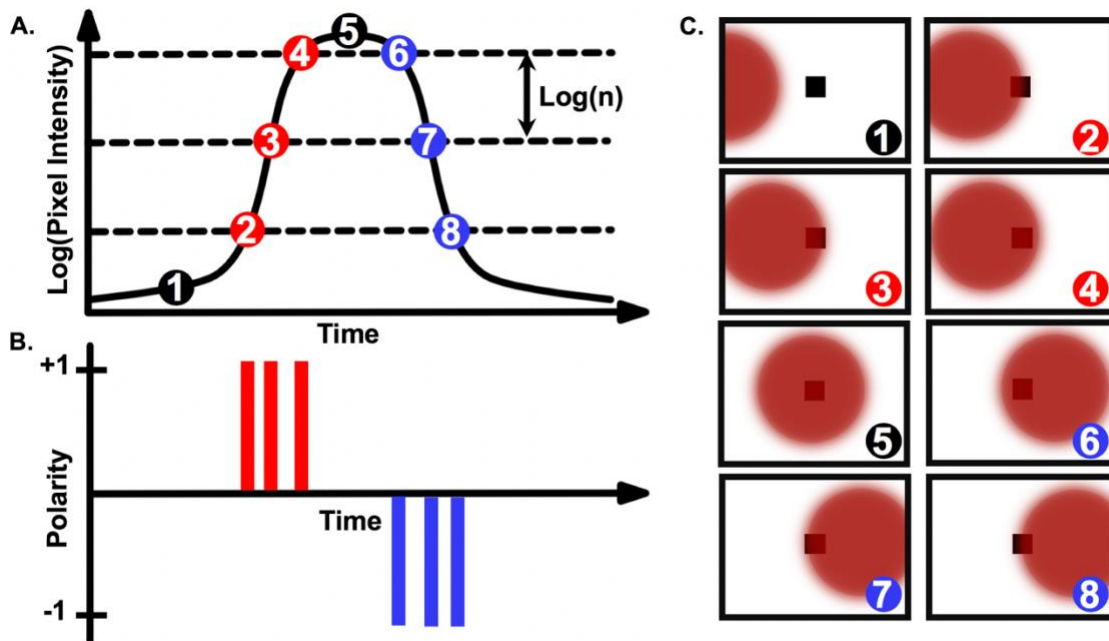
Visualising fluids and particles within channels is a key element of microfluidic work. Current imaging methods for particle image velocimetry often require expensive high-speed cameras with powerful illuminating sources, thus potentially limiting accessibility. This paper explores for the first time the potential of an event-based camera for particle and fluid behaviours characterisation in a microfluidic system. Event-based cameras have the unique capacity to detect light intensity changes asynchronously and to record spatial and temporal information with low latency, low power and high dynamic range. Event-based cameras could consequently be relevant for detecting light intensity changes due to moving particles, chemical reactions or intake of fluorescent dyes by cells to mention a few. As a proof-of-principle, event-based sensing was tested in this work to detect and track 1 μm and 10 μm -diameter particles flowing in a microfluidic channel. Importantly, experiments were performed by directly connecting the camera to a standard fluorescence microscope, only relying on the microscope arc lamp for illumination. We propose a data processing strategy that allowed particle tracking in both bright-field and fluorescence imaging. Tracking was achieved for particle velocities up to 0.4 $\text{m}\cdot\text{s}^{-1}$ demonstrating that event-based cameras could be a new paradigm shift in microscopic imaging.

Introduction

Event-based cameras emerged in the 1990s as neuromorphic vision sensors mimicking biological retinas (1). Unlike frame-based cameras, event-based cameras respond to, and only record, brightness changes (log intensity depicted as $\log(n)$ in Figure 1-A), asynchronously and independently for each pixel. When a change in brightness is detected at a given pixel, the event information is transmitted, that is, its (x,y) location on the pixel array, its time stamp and the sign/polarity of the change (increase (+1 in Figure 1-B) or decrease (-1 in Figure 1-B)).

Using current event-based cameras (available under 5k USD), events are detected with microsecond resolution. Since only events are transmitted, event-based cameras offer low latency, low power (ca. 10 mW) and high dynamic range (>120 dB) (2). These “silicon retinas” have become increasingly popular for high-speed robotic vision, e.g., for ball detection

48 (3)(4)(5), gesture recognition (6)(7), 3D mapping (8) or for unmanned aerial vehicles (9) and
 49 predator robots (10). They have also been used for tracking macroscopic objects such as
 50 vehicles (11) or stars/satellites (12)(13) (see the recent survey (28) which discusses
 51 applications and challenges of event-based sensing). Importantly, application to the “micro-
 52 world” is limited. The work proposed in (14) uses event-based cameras for micro-robotics,
 53 demonstrating tracking of microparticles in a petri dish while (15) focuses on neural activity
 54 imaging. In (16), particle tracking in a fluid-solid system has also been tested in a 5 cm ID pipe
 55 with 950 μm particles. The potential of event-based sensing for microfluidic applications
 56 remains consequently untapped.
 57



58
 59 **Figure 1.** Event-based detection of a particle over time. **A.** represents the light flux reaching
 60 a given pixel when a fluorescent particle passes through the pixel field of view. **B.** When the
 61 light intensity change (with respect to the last recorded event) exceeds a user-defined
 62 threshold, the camera records a new event whose polarity encodes the sign of the intensity
 63 change. **C.** Particle passing through the pixel field of view, generating light intensity changes
 64 depicted in A.

65
 66 Due to their characteristic microscopic scale, microfluidic systems primarily rely on imaging
 67 technologies (such as microscopes and cameras) to monitor fluids and particles inside a
 68 channel. Imaging modalities offer considerable flexibility and find applications in quality control
 69 (e.g., detection of dust/bubble), performance evaluation (e.g., mixing, separation, detection)
 70 or in better understanding localised phenomena but limitations remain. Let’s consider inertial
 71 microfluidics for example.

72
 73 Inertial focusing devices have been widely used by the community for their unique capabilities
 74 to focus and separate particles based on size, shape and/or deformability (17). Despite
 75 advances in the field of computational inertial microfluidics (18), prototypes are often tailored
 76 to a targeted application following long design/test/optimize iterations to empirically explore
 77 the capabilities of new channel designs. Accurately imaging fluid and particle behaviour has
 78 become essential to assess the underlying physical phenomena and inform further design

79 changes. Imaging inside inertial focusing devices (and microfluidics channels in general)
80 typically relies on either long-exposure fluorescence or high-speed imaging.

81

82 Long-exposure fluorescence consists of imaging fluorescent particles over an extended period
83 of time (long enough to be representative of the particle/fluid behaviour) and of building
84 corresponding composite images (by stacking/integrating several images). This approach is
85 particularly well suited to inertial devices with clear visualisation of “streaks” representing
86 fluorescent particles that are focused at specific locations inside the channel. By mapping the
87 intensity profile, an estimation of the focusing efficiency can be obtained. Long-exposure has
88 been used with a wide range of particles (e.g., beads (19)(20)(21)(22)(23), cancer cells (24),
89 bacteria (25)(26)(27) or fungal cells (28) to mention a few) and has enhanced our
90 understanding of the impact of channel geometry on focusing (20)(21)(29)(30). Advantages
91 of this imaging approach include a compatibility with standard fluorescent microscopes
92 equipped with conventional cameras and relatively little data processing. Importantly however,
93 the information extracted is reduced to global (*i.e.*, population) behaviours. Moreover, high
94 particle concentrations are usually required to ensure detectable fluorescent signals, hindering
95 an in-depth understanding of single particle behaviour.

96

97 On the other hand, high-speed imaging unveils other aspects of inertial focusing devices such
98 as the formation of trains of particles (31), the measurement of migration velocities (32) or the
99 number of focusing positions (29). In contrast to long-fluorescence imaging, studies exploiting
100 high-speed imaging for in-depth quantification are scarcer. One reason might be the
101 requirement of bespoke and expensive imaging systems to limit motion blur. Typically,
102 imaging can be performed with a high-speed camera synchronised with a high-power, pulsed
103 illumination source to reach exposure times in the order of 1 – 10 μ s (32)(33)(34)(35). Although
104 products have been commercialised e.g., by Dolomite, Fluigent or PreciGenome to offer plug-
105 and-play solutions to the community, such products are often limited to brightfield imaging. In
106 the presence of mixed populations, as often occurs in microfluidic systems, detecting and
107 differentiating particles without fluorescent signals can be a challenge. New approaches are
108 emerging to unlock the potential of high-speed fluorescence and/or 3D imaging (36)(37)(38);
109 however, micro-particle image velocimetry (μ PIV) remains the most widely accessible
110 technique. In μ PIV, the illumination is provided by a high-power, pulsed laser to record pairs
111 of images with a short time delay. μ PIV set-ups or similar have been used for inertial focusing
112 systems to access particle or fluid velocities (31)(32)(39)(40)(41)(42)(43). Access to μ PIV can
113 be challenging due to high capital cost; commercialised μ PIV set-ups are also often limited to
114 one wavelength, thus requiring iterative measurements for mixed populations (one
115 measurement per fluorescent population).

116

117 In this work, we investigate the potential of event-based cameras as a cost-effective
118 alternative to particle detection and tracking in microfluidic devices that 1) is compatible with
119 standard microscopes, 2) does not rely on high-power pulsed illumination sources, 3) is
120 significantly less data-consuming and less expensive than traditional, frame-based cameras
121 and 4) is attractive for both bright-field and fluorescence imaging. As proof-of-principle, particle
122 detection and tracking were performed in an inertial focusing device in both fluorescence and
123 bright-field modes for 1 μ m and 10 μ m-diameter beads. For the first time, this work reveals
124 the unique capabilities of event-based sensing for overcoming some of the commonly
125 encountered challenges in microfluidics imaging.

126

127 **Materials and Methods**

128 **Bead preparation**

129 Red fluorescent polystyrene beads, 10 μm or 1 μm in diameter (Magsphere) were diluted in
130 filtered phosphate-buffered saline (PBS) supplemented with 0.1% v/v Triton X-100 to a final
131 concentration of $1 \times 10^4 - 5 \times 10^5$ beads mL^{-1} . Beads concentrations were determined using
132 a haemocytometer.

133

134 **Microfluidic setup**

135 A spiral device was fabricated by lithography (Epigem, UK) and consisted of a channel in an
136 Archimedean spiral, with a rectangular cross section of 360 μm (width) x 60 μm (height). The
137 channel had one inlet, four outlets and 6 loops (Figure 2). Samples were injected into the spiral
138 channel *via* the inlet with a mid-pressure syringe pump (neMESYS 1000N, Cetoni, Germany)
139 and 1/16" polytetrafluoroethylene tubing with an internal diameter of 0.5 mm. Applied flowrates
140 in this work ranged from 0.05 to 2 $\text{mL}\cdot\text{min}^{-1}$ corresponding to Reynolds numbers in the interval
141 4 – 159; the Reynolds number is defined as $\text{Re}=\rho\text{UD}_h/\mu$, where ρ is the fluid density, μ is the
142 fluid viscosity, U is the velocity of the fluid and D_h the hydraulic diameter of the channel.

143

144 Prior to any measurement, and in between samples, 5 mL filtered PBS was flushed through
145 the syringe three times, and then through the spiral at 1.5 $\text{mL}\cdot\text{min}^{-1}$, to clean the system.
146 Experiments were performed in triplicate.

147

148 **Imaging setup**

149 Particles flowing in the spiral were characterised by capturing video footage (15 seconds
150 duration, from triplicate experiments) using two separate cameras. An event-based camera
151 (CSD3SHCD, Prophesee) consisting of a 480 x 360 pixels CMOS vision sensor, 20 μm x 20
152 μm event-based pixels and >10k frames per second (fps) typical equivalent frame rate, was
153 mounted on a Zeiss Axioskop 2 fluorescence microscope (Zeiss, Germany) to visualise the
154 spiral at 10X magnification. Prophesee player software (version 1.4.1-1935316) was used to
155 adjust the camera settings and to record videos. To validate data from the event-based
156 camera, a DinoLite camera (Dino-Lite) with a resolution of 1280 x 1024 pixels and a frame
157 rate of 30 frames per second (fps) was mounted on a clamp stand and positioned above the
158 spiral channel, and images recorded at ~50X magnification, 30 fps using DinoCapture 2.0
159 software.

160

161 Data analysis was performed using a bespoke data processing pipeline implemented in
162 Matlab, which is detailed in the following section.

163

164 **Event-data collection and pre-processing**

165

166 As mentioned above, event-based cameras do not provide series of frames but, instead, lists
167 of time-tagged events. Thus, pre-processing steps are required prior to information extraction.
168 As explained in Figure 1, events are recorded when the intensity change exceeds a user-
169 defined threshold. If this threshold is set too small, a large number of events are recorded,
170 including signal events but also spurious events considered as “nuisance events”. For
171 extremely low threshold values, this can lead to read-out issues whereby not all the events
172 can be properly recorded and transmitted. Conversely, using a large threshold value reduces
173 the number of background events but also the number of signal events, potentially hindering
174 the detection of particles generating intensity changes. Consequently, this threshold has to be

175 set carefully. Here, the trade-off between accurate particle detection and low background
176 noise was found *via* visual inspection using the camera software for each Reynolds number
177 and illumination mode. Note that, in the bright-field mode, the intensity changes are weaker
178 than in the fluorescence mode, leading to lower thresholds in practice.

179

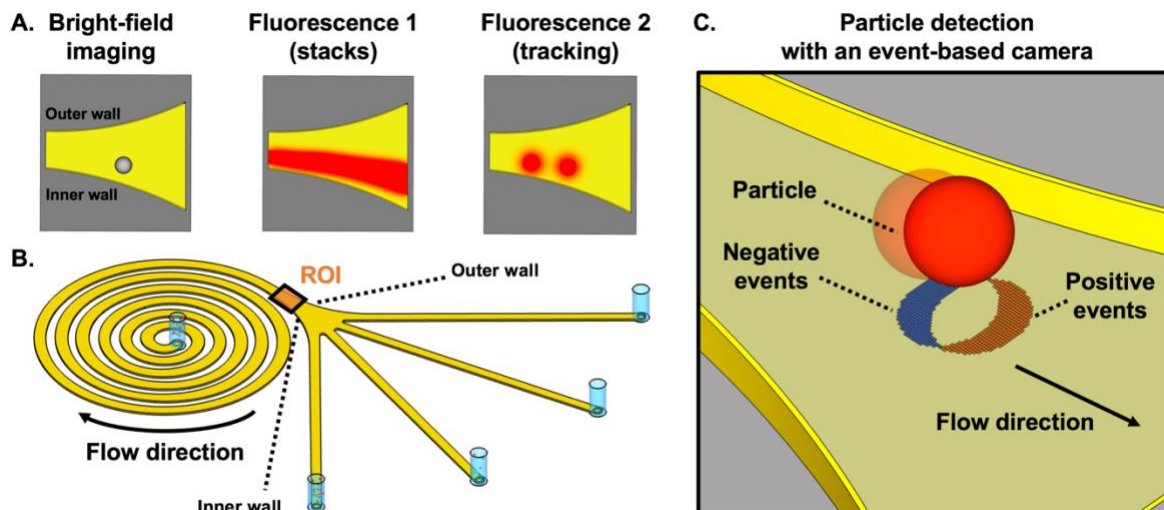
180 In principle, particle detection can be performed directly from the stream of detection events.
181 However, since event-based cameras are still an emerging technology, it is currently easier
182 to map the recorded events onto temporal frames and use mature image processing tools to
183 perform the detection task. This is the approach adopted here. One of the main advantages
184 of event-based cameras is that this frame-based representation can be obtained after the data
185 acquisition, and the frame rate, as well as the exposure, can be user-defined. Each pixel is
186 subject to a dead time, *i.e.*, a period of time after each detection, during which that pixel is not
187 able to record subsequent events. This dead time varies depending on the overall number of
188 events recorded in the array but is typically lower than 50 μs with the camera used here. Thus,
189 integration periods smaller than this value are not recommended. In the context of event-
190 based cameras, we identify the integration period as the temporal window used to aggregate
191 events and build a frame. Conversely, the longer this integration period, the blurrier the
192 reconstructed frames. Here exposures in the 100 μs – 1 ms range were used, depending on
193 the expected particle velocity and illumination mode, to find the best visual trade-off between
194 satisfactory particle detection and motion blur. Note that within a frame or integration period,
195 several events can be recorded by the camera at a given pixel. In that case, only the most
196 recent event is considered to generate the frame. The frame rate is also user-defined and can
197 be set independently from the per frame integration time, *e.g.*, it is possible to consider
198 overlapping integration windows. Again, setting this parameter can depend on the expected
199 speed of particles to be tracked. Low frame rates can lead to large distances travelled by the
200 particles between two frames and can jeopardize the tracking task, especially when multiple
201 particles are present in the field of view simultaneously (a challenging data association
202 problem). High frame rates make the tracking task easier, but might unnecessarily increase
203 the number of frames to be processed. In this work, the frame rate has been set to 20k fps, to
204 ensure the particles are visible in a sufficient number of frames to estimate their velocity (> 40
205 frames).

206

207 **Results and discussion**

208 As presented in Figure 2, the passage of spherical particles through a spiral channel with a
209 rectangular cross-section (360 μm x 60 μm) was analysed with an event-based camera.
210 Particle detection and tracking were performed near the outlet of the spiral (1200 μm x 1500
211 μm region of interest (ROI) in Figure 2-B).

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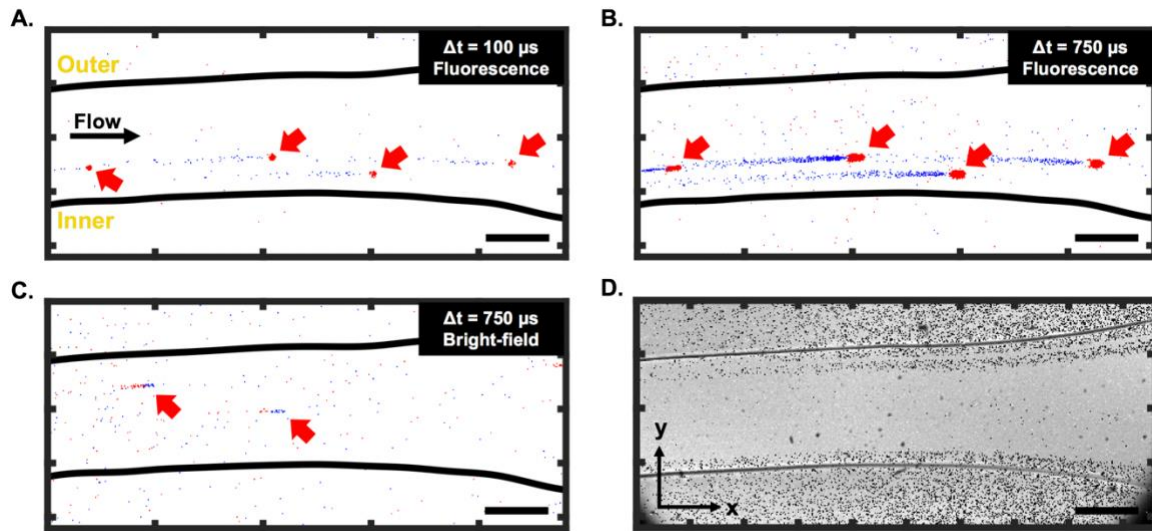
Figure 2. *Imaging in inertial focusing devices. A. Standard approaches for imaging particles in inertial focusing devices include bright-field (or phase contrast) images of moving particles, composite images from long-exposure fluorescent signals or single fluorescent particle visualisation (e.g., using particle image velocimetry). B. Schematic of the microfluidic device tested in this work; ROI: region of interest. C. An event-based camera was used for particle detection and tracking in the ROI. Negative (blue) events correspond to pixels detecting a decrease in brightness and positive (red-brown) ones to an increase.*

222 Based on previous work with similar designs (44)(45)(46), 10 μm rigid spherical beads were
223 expected to focus towards the inner wall of the spiral channel for Reynolds numbers above
224 ~ 50 in the region of interest of the channel, while 1 μm particles were expected to remain
225 unfocused. It can also be noted that according to (47), no secondary Dean vortices are
226 expected for the Reynolds numbers in the range of interest ($\text{Re} < 160$). With an average fluid
227 velocity in the spiral of 0.5 $\text{m}\cdot\text{s}^{-1}$ at $\text{Re} = 50$, a light source with a very short pulse duration
228 ($\sim 10 \mu\text{s}$) would normally be necessary for single particle detection to circumvent motion blur.
229 In this work, only a standard lighting source (Zeiss HBO100 Mercury vapor short-arc lamp)
230 from a fluorescent microscope was used with the event-based camera.

231 **Visualisation and detection of microparticles**

232 As visible in Figures 3-A and -B (and video in the supplementary information), 10 μm
233 fluorescent particles can be clearly seen at a low Reynolds number ($\text{Re} = 4$) using the event-
234 based camera. The motion of particles in bright-field mode (Figure 3-C) was also
235 demonstrated, although the contrast is generally lower than in the fluorescence mode. Figures
236 3 A-C highlight one specific characteristic of event-based cameras, namely the possibility to
237 define, after the data recording, the integration period and frame rate for visualisation/analysis
238 purposes, as discussed above. Although not all event-based cameras provide this feature, the
239 camera used in this work also records information allowing the reconstruction of grey scale
240 images (but at a much lower time resolution than the event-data processed here). This feature
241 was used to create a long-exposure intensity image (in bright-field mode) for calibration
242 purposes and to identify the location of the channel in the field of view. An example is depicted
243 in Figure 3-D.

244



246

247 **Figure 3.** Raw data for 10 μm particle visualisation inside the spiral channel at $Re = 4$ in
 248 fluorescence mode for an integration time of 100 μs (A) and 750 μs (B). C. Example of raw
 249 data for bright-field imaging with the event camera and an integration time of 750 μs . Red
 250 arrows denote the position of particles in the channel, red pixels correspond to pixels detecting
 251 an increase in brightness and blue ones to a decrease. D. Reconstructed long-exposure
 252 intensity image. The scale bars (in the bottom right corner of each subplot) correspond to 200
 253 μm , the inner and outer walls are defined in A.

254

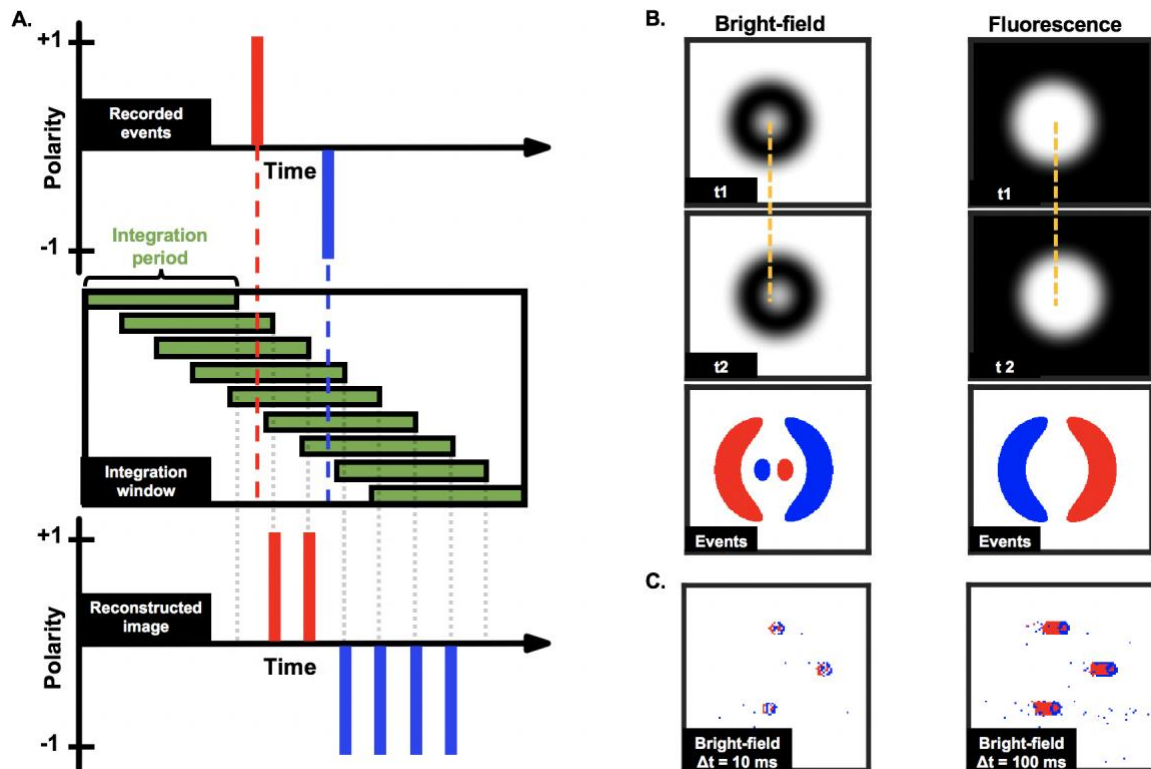
255 It is interesting to note from Figure 3 that the trails produced by moving particles (e.g., the
 256 negative (blue) events for fluorescent particles in Figures 3 A-B) are usually longer than the
 257 events created when the particles first become visible in a given pixel. As presented in Figure
 258 4-A, this phenomenon is due to the fact that only the most recent event is kept at each pixel
 259 when constructing the frames. Depending on the selected integration period, the
 260 reconstructed image consequently has more negative (blue) events than positive (red) ones
 261 for fluorescent particles (and more positive events for bright-field reconstructed images).

262 Figure 3 also shows that moving fluorescent particles induce a local increase of light flux, while
 263 particles in bright-field mode are characterized by a local reduction of the light flux.
 264 Consequently, fluorescent particles first produce positive events and then negative events,
 265 depicted in red and blue, respectively, in Figures 3-A and -B. Conversely, particles in bright-
 266 field first produce negative events and then positive events (Figure 3-C). This detail is essential
 267 to process data appropriately and differences between bright-field and fluorescence imaging
 268 are further highlighted via the simulated data presented in Figure 4-B.

269

270 In bright-field mode, static particles typically appear as dark rings (48). The darker edges and
 271 lighter centre can create a characteristic pattern on the reconstructed images (see simulated
 272 images in Figure 4-B, left panels), especially for slowly moving particles. Despite not being
 273 visible in Figure 3-C due to the high velocity of the particles, this pattern was observed at
 274 higher magnification (20x) and lower particle velocity ($\sim 0.0003 \text{ m.s}^{-1}$), as reported in Figure 4-
 275 C. It can also be noted that for slow-moving objects, the length of the tail could be used to
 276 estimate the particle velocity from a single image, *i.e.*, without the need for advanced tracking

277 algorithms. However, Figure 3 shows that the length of the tail of high-velocity particles cannot
 278 be accurately measured due to the low signal-to-noise ratio in that case.
 279



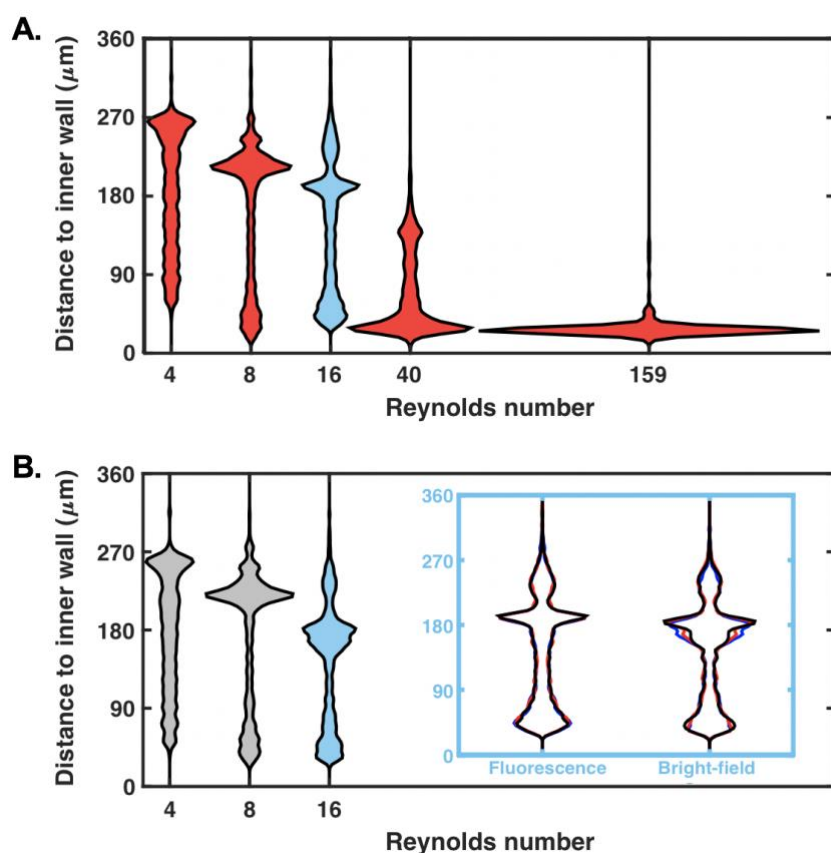
280
 281 **Figure 4. A.** Example of image reconstruction (single pixel presented here) from recorded
 282 asynchronous events. The events are mapped onto temporal frames based on a user-defined
 283 integration window. This gives access to a sequence of reconstructed images that can then
 284 be analysed using image processing tools. The event camera only keeps information
 285 corresponding to the last event, leading to “trails” (higher number of negative (blue) events in
 286 this example) on the reconstructed image. **B.** Simulation of moving particle between times t_1
 287 and t_2 in bright-field and fluorescence modes and corresponding events. The third row from
 288 the top illustrates how the (simulated) reconstructed event frames are expected to look like
 289 (for a sufficiently short time delay t_2-t_1) and short integration time. The displacement of the
 290 particles is further highlighted by the dashed yellow lines. **C.** Reconstructed images obtained
 291 from measurements of $10\ \mu\text{m}$ particles slowly moving in the spiral channel for an integration
 292 time of 10 ms and 100 ms. As in Figure 3 A-B, the longer the integration time, the longer the
 293 trails.

294
 295 Consequently, in the following sections, only the positive events are used to detect particles
 296 in fluorescence, and respectively, negative events to detect particles in bright-field mode. More
 297 precisely, the frames of positive or negative events are first denoised using morphological
 298 transforms (e.g., erosion and dilation) to remove isolated events. The particles are then
 299 extracted by identifying groups of spatially connected events whose size falls into a predefined
 300 range. The position of the particle is then computed as the position of the centroid of each
 301 region. Note that here, all the particles used had the same apparent size, but the size/shape
 302 of the connected regions could be used in the future to classify particles and potentially
 303 enhance the tracking results.
 304

305 **Quantification of focusing behaviours in fluorescence and bright-field modes**

306 In addition to demonstrating that 10 μm particles can be detected at low Reynolds numbers,
 307 we investigated whether detection was possible for the range of Reynolds numbers which are
 308 usually considered for focusing experiments with such microfluidic designs. The spatial
 309 distribution (expressed as the distance to the spiral channel inner wall) of particles detected
 310 next to the outlet of the channel, in a region of constant cross-section (before the opening),
 311 was recorded at different flow rates (Figure 5-A). For visualisation purposes, violin plots were
 312 used as a comprehensive representation of the spatial distribution of particles. A wide
 313 horizontal spread of these plots (normalised by the number of detected particles in each case)
 314 corresponds to a large number of particles detected within a narrow region of the channel
 315 (characteristics of focusing).

316



317
 318 **Figure 5. A.** Distribution of particles detected in one video recorded with an event-based
 319 camera using fluorescence microscopy for Reynolds numbers $Re = 4, 8, 16, 40$ and 159 ,
 320 corresponding to applied flow rates in the interval $0.02 - 2 \text{ mL}\cdot\text{min}^{-1}$ and averaged fluid
 321 velocities in the interval $0.04 - 1.54 \text{ m}\cdot\text{s}^{-1}$. **B.** Distribution of particles detected with an event-
 322 based camera using bright-field microscopy for Reynolds numbers $Re = 4, 8, 16$. **Inset.** For
 323 $Re = 16$ (blue plots), three replicates (blue, red and black) are superimposed in the inset for
 324 both fluorescence and bright-field imaging. All the distributions depicted in this figure are
 325 normalized such that they do not depend on the particle concentration. Thus, all the violin
 326 plots (except in the inset which has presents a different vertical scale) have the same area.
 327

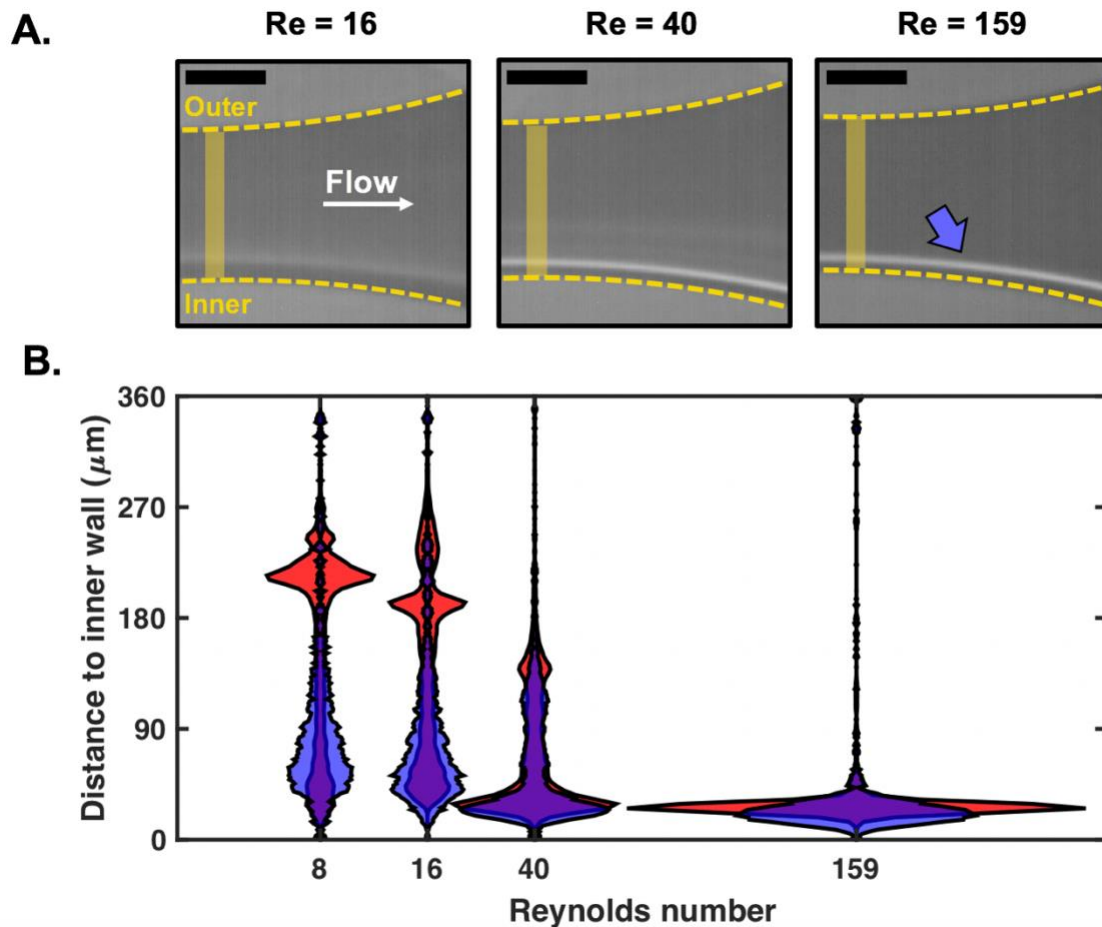
328 Particles were successfully detected in fluorescence mode for $Re \in [4,159]$. The focusing of
329 beads close to the inner wall is clearly visible when the Reynolds number increases, as
330 expected for this microfluidic design (45). Note that each distribution has been derived from
331 at least 1000 particles and that similar distribution profiles can be plotted at any x-location
332 along the channel imaged (*cf.* Figure 3-D for definition of x axis), or mapped onto the entire
333 section of the channel imaged (the channel length that is imaged with the current set-up is
334 circa 1.5 mm).

335 For comparison purposes, similar experiments were performed in bright-field mode. Due to a
336 lower contrast between beads and background, reliable detection was only possible up to Re
337 = 16 (Figure 5-B). To measure the similarity between the distributions obtained in Figures 5-
338 A and -B, we computed their percentage overlap. For $Re = 4, 8$ and 16, the overlap between
339 the distributions plotted in Figures 5-A and -B was 88%, 79% and 77%, respectively. The
340 distributions of fluorescence and bright-field modes are generally in good agreement and the
341 increasing discrepancy with increasing Re can be partly explained by a degradation of the
342 detection performance in bright-field mode.

343
344 With an average fluid velocity of $0.2 \text{ m}\cdot\text{s}^{-1}$ at $Re = 16$, being able to detect particles without a
345 pulsed light confirms the potential of event-based cameras for particle detection in microfluidic
346 channels in both fluorescence and bright-field modes. Note that 1) results for both
347 fluorescence and bright-field modes were highly reproducible as demonstrated for three
348 replicates at $Re = 16$ (*cf.* blue inset, Figure 5-B; overlap > 94% for replicates in fluorescence
349 mode and >89% in bright-field mode) and 2) detection at higher Reynolds number might
350 possibly be achieved upon further optimisation of the hardware/software (see discussion in
351 conclusion).

352
353 As a control, long-exposure fluorescence imaging was used for all the Reynolds numbers
354 considered (*cf.* Figure 6-A) – in this case, images were recorded at 30 fps and stacked over a
355 15 second period. Fluorescence intensity distributions were then estimated in a measurement
356 window similar to the one used in Figure 5. As visible in Figure 6-B, the intensity distributions
357 follow patterns and trends similar to those obtained using the event-based camera, with
358 focusing occurring at the inner wall of the spiral channel with increasing Reynolds numbers.
359 The horizontal spread of the fluorescence distribution at $Re = 159$ does not appear as sharp
360 as the particle distribution, which might be due to pixel saturation and the likely non-linear
361 relationship between the particle and light intensity densities. Only one main mode is also
362 observed in Figure 6-B for long-fluorescence imaging data, while two streams of focusing
363 seem to be detected at lower Reynolds numbers with the event-camera. Multiple focusing
364 streamlines in inertial devices have been previously reported in the literature, especially for
365 higher volume fractions (44,49). However, the particle concentration used here has been kept
366 the same for event-based and long fluorescence imaging experiments. Although the exact
367 nature of this observed second mode remains unclear; its observation for both event-based
368 fluorescence and bright-field modes seems to confirm that this is not an artefact from the
369 cameras or from the data processing pipeline.

370



371
372

373 **Figure 6. A.** Composite images of 10 μm fluorescent beads flowing near the outlet of a spiral
374 channel at $\text{Re} = 16, 40$ and 159 . The dashed lines correspond to the channel walls, the yellow
375 rectangles are regions where the intensity distributions have been estimated and the arrow
376 highlights the focused stream of beads at $\text{Re} = 159$. Scale bars: 200 μm . **B.** Intensity
377 distributions estimated in the measurement windows (yellow rectangles in **A**) and depicted as
378 distances to the channel inner wall in blue. Particle densities obtained from the event-camera
379 are superimposed in red. All the distributions depicted in this figure are normalised such that
380 all the violin plots have the same area.

381

382 **Particle tracking and velocity mapping**

383

384 In this section, the potential of event-based imaging for particle tracking is investigated. Only
385 results in fluorescence mode are reported here but tracking was also achieved in bright-field
386 mode for all the Reynolds numbers reported in Figure 5-B.

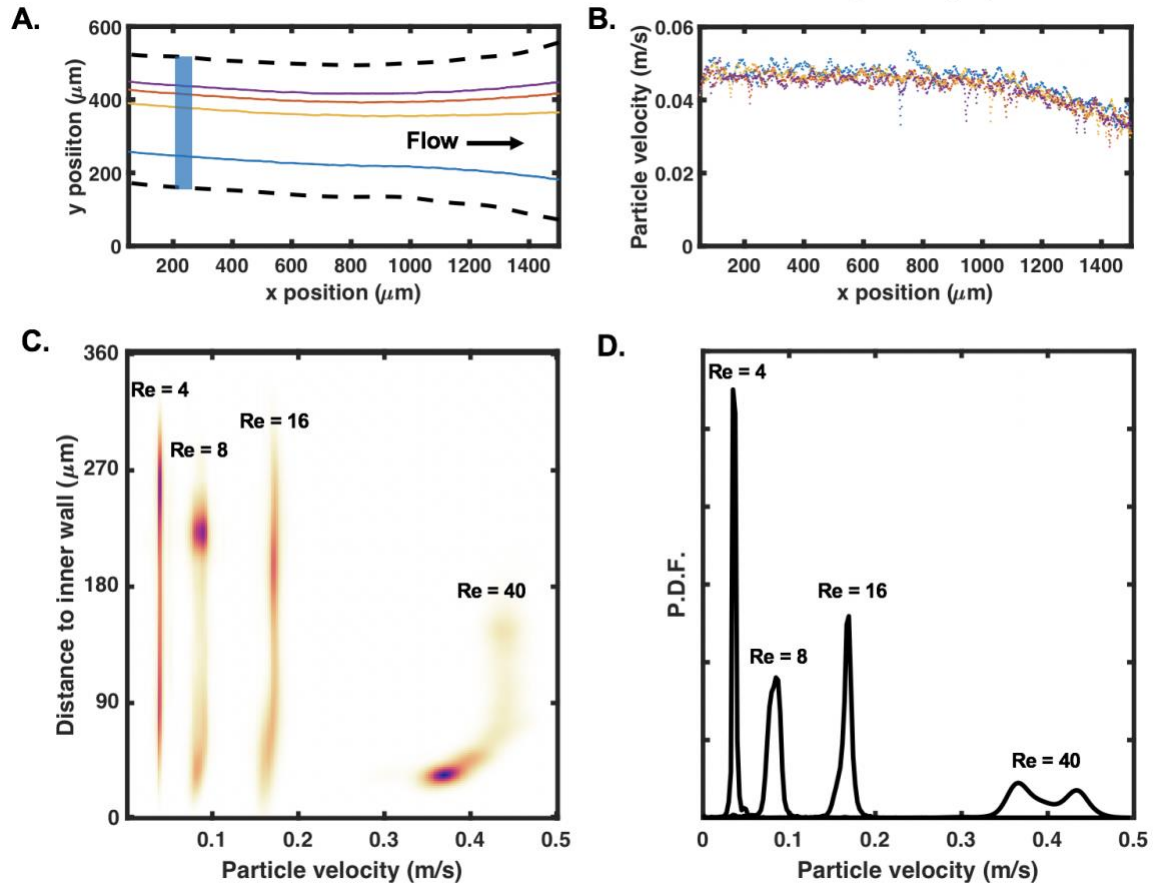
387

388 Depending on the concentration of particles, a varying number of particles can be observed
389 simultaneously in the field of view. Consequently, algorithms for multiple target tracking (MTT)
390 can be used. Although it might be possible to identify particles from the complete set of
391 generated frames, in practice, it is computationally intractable, given the high frame rates
392 considered (20M frames for a 10 s experiment). In all experiments performed, the number of
393 particles simultaneously present was relatively low (less than 10) and the particles presented
394 similar trajectories and velocities. Thus, a standard online approach to MTT was adopted,

395 which updates the particle tracks sequentially as each frame is processed. Following the
396 particle detection steps described above, the data association problem was solved using a
397 variant of the Munkres algorithm (50)(51). This problem consists of deciding which detected
398 particles are associated with existing tracks (from previous frames) and which are new
399 particles. Once the data association is performed, the actual tracking of each particle is
400 performed using a standard Kalman filter (52) assuming a near-constant velocity model (for
401 each track). The algorithm also includes a track ending mechanism, which terminates tracks
402 for particles that have not been seen over a given period. This makes the algorithm more
403 robust against missed particles (which might not be detected in a few frames during the
404 detection process).

405

406 Figure 7-A presents an example of 4 tracks of 10 μm particles identified in the channel at Re
407 = 4 (one colour per track), leading to the estimated particle velocities plotted in Figure 7-B. An
408 example of tracking is also visible in the video (*cf.* supplementary information). The estimated
409 average particle velocity of 0.05 $\text{m}\cdot\text{s}^{-1}$ is in accordance with an average fluid velocity of 0.04
410 $\text{m}\cdot\text{s}^{-1}$. A slight decrease in particle velocity can be observed when approaching the outlets (x
411 > 1000 μm), as expected due to the opening of the channel. Particle velocity profiles were
412 then plotted as a function of the distance to the inner wall for different increasing Reynolds
413 numbers. As presented in Figure 7-C, successful tracking was achieved up to $\text{Re} = 40$; the
414 colormap in this figure highlights in purple, regions where many detected particles present
415 similar velocity and distance to the inner wall, for a given Reynolds number. For $\text{Re} < 40$, an
416 approximately constant velocity is measured for all the particles tracked (>1000 for each
417 experiment). For particles detected closer to the inner wall, especially at $\text{Re} = 40$ with a high
418 number of particles tightly focused in the region near $y = 45 \mu\text{m}$, a decrease in particle velocity
419 is observed. This behaviour in particle velocity is further confirmed by the probability density
420 functions displayed in Figure 7-D.



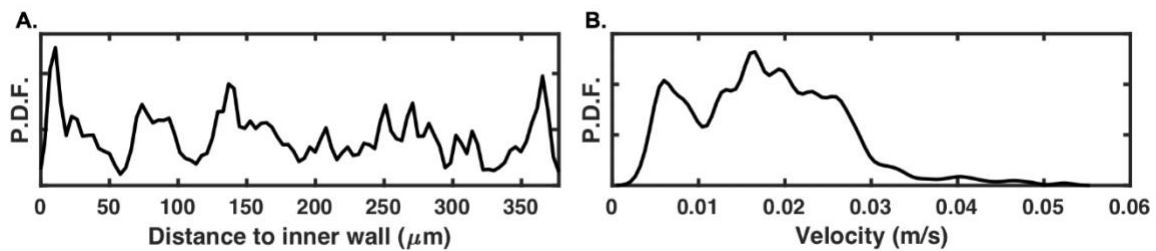
421
422

423 **Figure 7. A.** Example of tracks for four randomly picked 10 μm particles flowing in the spiral
424 at $\text{Re} = 4$ (one colour per track). Dashed lines correspond to the microfluidic channel. Each
425 track contains approximately 800 positions. **B.** Corresponding estimated particle velocity (raw
426 data) as a function of the x-position in the channel. **C.** Particle velocity profile as a function of
427 the distance to the inner wall for $\text{Re} = 4-40$ and **D.** corresponding probability density functions
428 (P.D.F.). Plots in C are based on particles tracked in the blue region of interest highlighted in
429 **A.** Colormap highlights particle density, with dense regions in purple (linear colour map L17 in
430 (53)).

431

432 The results in Figure 7 confirm that event-based cameras can be used to track individual
433 particle behaviours in the size range of commonly used biological cells. In order to map fluid
434 patterns, however, smaller sizes would typically be used for μPIV experiments. Experiments
435 were consequently also conducted with 1 μm fluorescent particles at $\text{Re} = 4$. With the current
436 set-up, one pixel of the 480 x 360 pixels CMOS vision sensor corresponds to a $\sim 3.3 \mu\text{m} \times 3.3$
437 μm field of view. As presented in Figure 8-A, a subpixel detection was possible in fluorescence
438 mode, with 1 μm particles successfully detected across the channel. The estimated probability
439 density function of particle velocity is displayed in Figure 8-B. With an average fluid velocity of
440 0.04 $\text{m}\cdot\text{s}^{-1}$ it seems that only particles with lower velocities ($< 0.03 \text{ m}\cdot\text{s}^{-1}$) were successfully
441 detected and tracked. Due to the small size of the particles, the fluorescence-induced intensity
442 change was lower than with the previously tested 10 μm particles, causing a lower signal-to-
443 noise ratio while the particle passing time was shorter. Importantly, the fact that 1 μm beads
444 could still be detected and tracked illustrates further the potential of event-based sensing for

445 μ PIV experiments in microfluidic devices; a higher detection performance could be reached
446 by either increasing the magnification or working with sensors with a higher number of pixels
447 (cf. (2) for a descriptive review of existing cameras available and characteristics).
448



449
450 **Figure 8.** Tracking results of 1 μ m fluorescent particles at $Re = 4$. Probability density functions
451 (P.D.F.) of **A.** tracked particles as a function of the distance to the spiral inner wall and **B.**
452 corresponding particle velocities. Plots **A** and **B** are based on particles tracked in the blue
453 region of interest highlighted in Figure 7-A.
454

455 For both 10 and 1 μ m particles, no information was gained on the z-position of particles
456 detected at this stage; a similar approach to (32) using velocimetric reconstruction for
457 reconstructing the z-position could potentially be used with event-based data too.
458

459 Conclusions

460 Event-based cameras offer unique advantages to track high-speed phenomena thanks to their
461 sensors acting as silicon retinas. Although the benefits of this technology have been already
462 demonstrated for robotics, its potential for biological/microscopic applications remains largely
463 untapped. In this work, the performance of an event-based camera for detecting and tracking
464 micrometric particles in a microfluidic channel was evaluated. Inertial focusing devices, due to
465 their high working Reynolds numbers, are often recognised as challenging systems for
466 individual particle tracking. Current approaches typically rely on high power, pulsed
467 illumination sources and expensive micro-PIV setups to track fluorescent particles. The
468 present work demonstrates that event-based cameras can offer an alternative to such state-
469 of-the-art imaging setups. Particle detection was possible for a wide range of Reynolds
470 numbers, up to $Re = 159$, simply by using a standard fluorescence microscope (and lighting),
471 both in bright-field and fluorescence modes. As opposed to micro-PIV setups, the event-based
472 camera is not limited to one wavelength; any particles that are excitable in the visible spectrum
473 with the microscope can potentially be detected. Although more challenging to accurately
474 track, the velocity profile of particles down to 1 μ m was also measured with the tested set-up.
475 Since the application of event-based cameras to the microfluidic world is still new, specific
476 challenges/limitations also need to be considered. The field of event-based cameras is fast
477 evolving with always faster and more sensitive sensors being developed. For instance, efforts
478 are currently made to increase the fill-factor and reduce the pitch of event-based detectors,
479 and at the same time to produce larger arrays to improve the spatial resolution. However, it is
480 important to mention that as opposed to the camera tested here, most products do not directly
481 offer grey scale “reconstructed image”. This might cause significant difficulties to set up the
482 system (e.g., for the focus) since only moving/blinking objects are visible on display. This could
483 be tackled by engineering new tools to help with the calibration either in the setup itself or
484 computationally.
485

486 Finally, the drastic changes in data – from images to events – imposes the development of a
487 new framework for processing. Importantly, it has been demonstrated here that data can be
488 analysed to extract relevant information (e.g., particle focusing position, particle velocity) and
489 can also be directly compared to images (e.g., comparison with composite image from
490 fluorescent imaging). This, in addition to their high sensitivity to intensity changes,
491 compatibility with standard microscopes, high speed capabilities, low consumption and lower
492 costs compared to standard high-speed cameras, makes event-based cameras unique
493 candidates to change our way of characterising the microscopic world.

494

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496

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501

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504 YA and MJ wrote the manuscript; all authors discussed the results and commented on the
505 manuscript. YA and MJ equally contributed to this work.

506

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