Freeze Frame Imaging- a new imaging technique for fast dynamics particle tracking

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2023

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We here disclose an imaging technique called Freeze Frame Imaging (FFI) that enables recording of fast dynamics in acoustofluidics without the need for high-speed cameras or high-intensity light sources. In FFI the sound field is toggled on and off in sync with the frame acquisition which enables extended exposure times when particles are at rest and thus avoiding motion blur. We show that FFI maps velocity fields accurately compared to continuous operation at slow dynamics, that it allows for accurate recording of fast dynamics, and we explore the upper frame rate limit.

In acoustofluidics, measuring the acoustic mobility is important when determining the acoustic properties of an unknown cell suspension [1], as well as when characterizing new acoustofluidic devices [2]. The normal procedure for this is to use particle imaging velocimetry or particle tracking velocimetry to measure the migration velocity of particles within the acoustic field. However, acoustofluidics can produce strong forces on particles that lead to fast dynamics, which is challenging in these methods due to blurred particle images and typically require high speed cameras and powerful light sources. Even using specialized hardware, nm-sized particles or fluorescently labelled cells are very difficult to measure accurately because of the low amount of light they emit.

The principle behind the FFI method relies on pulsed acoustic actuation. The electronic circuitry actuating the acoustofluidic device is hardware synchronized with a camera (fig.1). In contrast to actuating the acoustic field continuously, the field is toggled on for a specific time, then toggled off again. An image is taken in between every actuation cycle when particles are stationary. This creates an artificially higher framerate when measuring mobility and additionally decouples the illumination time from the framerate.

Experimentally we measured the acoustic mobility of 5µm particles in a 2MHz acoustic standing wave, as described in (fig.3), with and without FFI (fig.2). The FFI measurement shows agreement with conventional imaging (80FPS) for slow dynamics up to 1mm/s. For faster dynamics above 1mm/s, conventional imaging tends to underestimate the velocity, whereas the FFI measurements follows the expected linear relation between voltage^2 and velocity. Our data shows that the framerate can be artificially increased from 80FPS to 6400FPS, a FFI multiplicator of 80, without significantly changing the acoustic field (fig.4), allowing mobility measurements of 10mm/s or higher. For higher FFI multiplicators than 80, (actuation pulses shorter than 150 µs), the acoustic field shows degradation and is not equivalent to a continuously actuated field, resulting in an upper FFI limit. Another benefit from the FFI is that it allows the use of a longer exposure time than conventional imaging with the same framerate. Since the imaged particles are stationary when the frame is taken, motion blurring is suppressed. A long exposure time also increases the signal to noise ratio (SNR) in the images (fig.5a-c).

Finally, we propose that this imaging technique is suitable for studying migration velocities in other actively driven microfluidic systems with low Reynolds numbers, such as magnetic, dielectric, and electrophoretic devices.

1. Barnkob, R., et al., *Measuring the local pressure amplitude in microchannel acoustophoresis.* Lab on a Chip, 2010. **10**(5): p. 563-570.
2. Qiu, W., T. Baasch, and T. Laurell, *Enhancement of Acoustic Energy Density in Bulk-Wave-Acoustophoresis Devices Using Side Actuation.* Physical Review Applied, 2022. **17**(4): p. 044043.

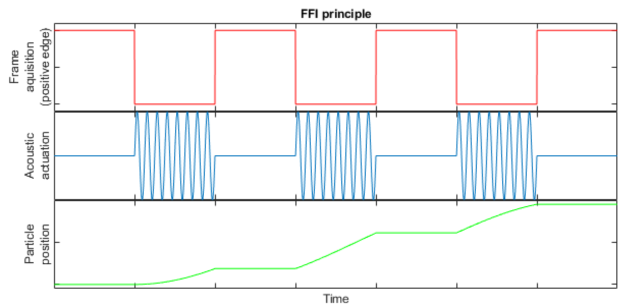

Fig 1. *An illustration showing the principle behind FFI imaging. The camera synchronization signal (red) controls the camera. The acoustofluidic device is actuated intermittently (blue), between frames. The particles only migrate when the device is actuated (green).*

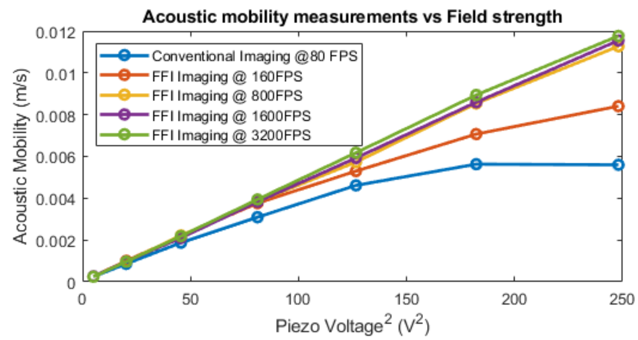

Fig 2. *Acoustic mobility measurement vs Piezo Voltage^2 (~field strength), using different framerates. Conventional imaging techniques (80FPS) is not suitable for motion tracking above 1mm/s, where as the FFI accurately can track particles in stronger fields. Each data point is an average of 20 experiments. The whole series consists of 700 experiments*

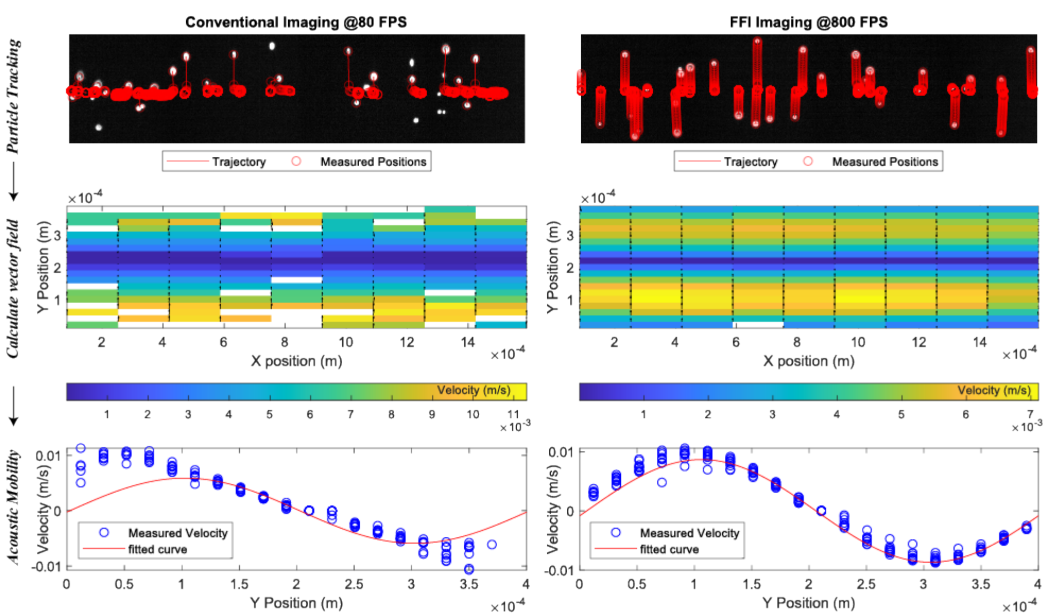

Fig 3. *Conventional imaging at 80 FPS (left) vs FFI at 800 FPS (right). The lower framerate of conventional imaging is not sufficient for particle tracking. The figure shows the three steps to measure acoustic mobility:*
1. Track particles.
2. Calculate a vector field
3. Calculate the acoustic mobility by fitting a sine.

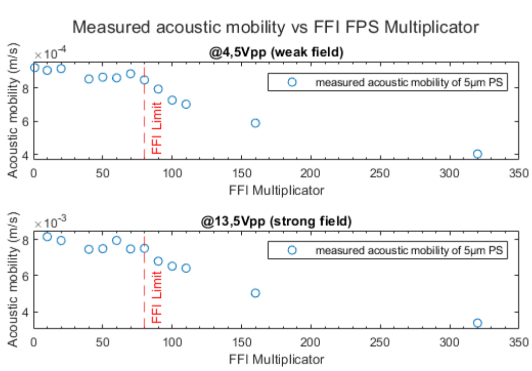

Fig 4. *Testing the limit of the FFI technique in acoustic fields with different strengths. The FFI can increase the artificial FPS (6400) a factor of 80. Above the FFI limit the acoustic field is nonequivalent to a continuously actuated field.*

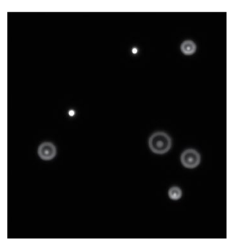

Fig 5a. *A long exposure time with FFI yields images suitable for particle tracking.*

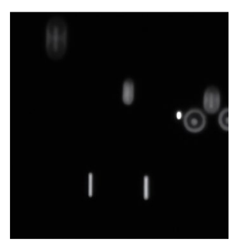

Fig 5b. *A long exposure time with conventional imaging results in motion blurring.*

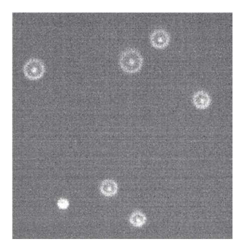

Fig 5c. *Short exposure time reduces motion blurring but gives images with low SNR.*