Supplementary Material (ESI) for Lab on a Chip

Supplementary Information for:

Sub-pixel resolving optofluidic microscope for on-chip cell imaging

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S1. Detailed calculation of the motion vector and an algorithm for motion estimation

Because the motion of a sample flowing within the microfluidic channel may not be perfectly uniform, techniques for estimating the motion of the sample using only the LR image sequence are necessary for the reconstruction of a HR image. While in some cases, the motion of a sample may be approximated as linear, Figure S1 shows the improvement of the HR image's quality from using a motion vector measured from the LR sequence using the methods described below, compared with the result from merely using a linear motion vector.

For highly scattering samples, we developed and used the following technique to generate a motion vector for a LR image sequence. First, the background of each LR image is subtracted and contrast enhanced. Highly scattering objects in LR images appear darker than the background, thus we can use this information to determine the location of the sample within each LR frame. To do this, we binarize the image using a single threshold computed from the entropy of the image histogram[1]. The geometric centroid of each of these binarized images from the LR image sequence is then computed and the entire array of centroids is low pass filtered to account for both the smooth flow pattern of the sample and the quantization error from binarizing and computing the centroid of each individual LR image.

For samples with lower contrast or bright spots, the motion vector is calculated in a similar manner, except the images are binarized with two thresholds. We calculate the background noise level and binarize the image by converting any pixel that is a certain amount above or below the noise level to 0 while making the rest

1. Also, in order to avoid errors due to the geometry of the sample, we use the pixel coordinate of the bounding box of the object and low pass filter the data to obtain sub-pixel shift values. For microspheres that are smaller than a pixel size, it is more difficult to distinguish a pixel response that results from the shadowing of the microsphere from the sensor's background noise. In this case, we obtain the motion vector by observing the blinking of the pixels on the expected pathway of microspheres (parallel to the channel wall). From the raw data, we can obtain the pixel coordinates where the microspheres are expected to flow through. This pathway can be any line of pixels within the channel that are parallel to the channel wall, since the microspheres follow the laminar flow of the solution. We then extract the time variation of the pixel response on the pathway, which shows the blinking of the pixels as the microsphere moves across each pixel, and calculate the motion vector from the slope ($\Delta x/\Delta f$, $\Delta y/\Delta f$).

These techniques that we developed for motion estimation, essential for reconstruction of a HR image, allow the motion of the sample to be estimated from only the LR image sequence. With this technique, the requirement for uniform and laminar flow or use of a precision scanning or stepping detector or light source is eliminated.

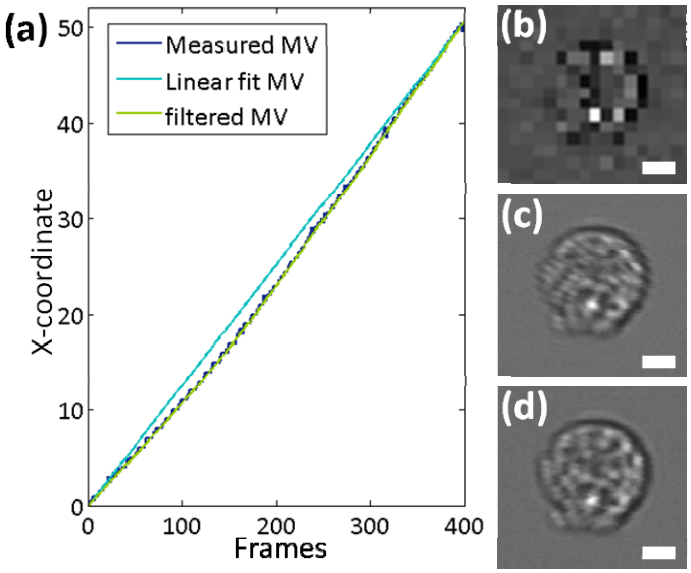

Figure S1. Improvement of the HR image with the non-linear motion vector estimation. (a) Measurement of motion vector from the raw sequence shows the error between the linearly estimated motion vector and the actual motion of the object. (b) The raw image of the sample. (c) HR image reconstructed with the linearly estimated motion vector. The streaks and jagged edges in the image result from the discrepancy between the motion vector used in reconstruction and the actual location of the object. (d) HR image reconstructed with the non-linear motion vector measured with the methods described above. All scale bars indicate 10μm.

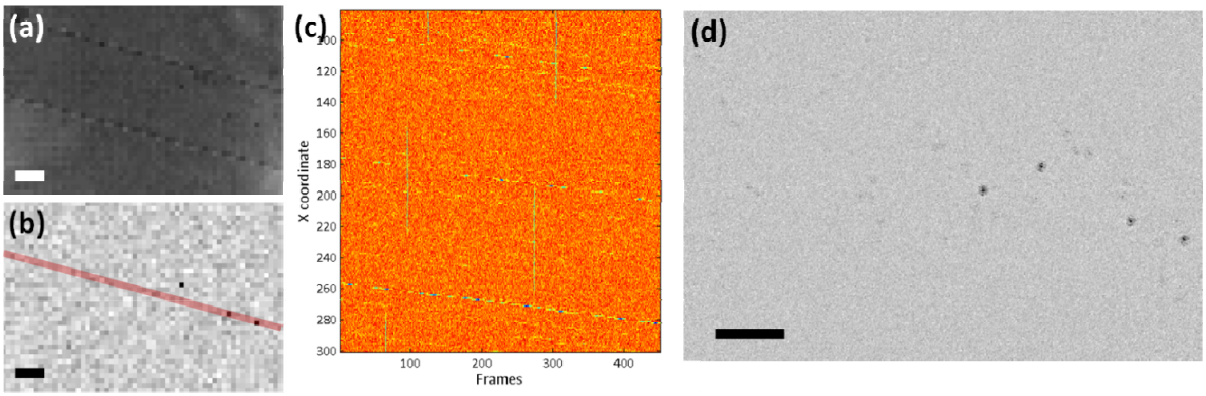

Figure S2. Motion estimation of microspheres smaller than the pixel size (1μm microspheres). (a) Raw LR image showing the channel structure. (b)Normalized raw image with the contrast enhanced. The response of the pixels under the red line, which is parallel to the channel wall, is processed to obtain the motion vector. (c) Time resolved response of the line of pixels show the microspheres passing through each pixel. The slope of streaks ($\Delta x/\Delta f$) indicates the motion vector in x direction and $\Delta y/\Delta f$ can be calculated from the slope of the red line in (b). (d) HR image of the microspheres reconstructed with the motion vector obtained from (c). All scale bars indicate 20μm.

References

1. J. Kapur, P. Sahoo and A. Wong, Computer vision, graphics, and image processing, 1985, 29, 273-285.