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Measurement Uncertainties in MEMS Kinematics by Super-Resolution Fluorescence Microscopy

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Introduction

Microelectromechanical systems (MEMS) have characteristic structural dimensions best measured in micrometers, but often exhibit nanoscale motions that are critical to system performance. Super-resolution fluorescence microscopy has been used to characterize the nanoscale kinematics of a standard MEMS actuator. Fluorescent nanoparticles are deposited onto the MEMS device, providing a constellation of near-ideal point sources of light. The constellation is imaged with a widefield epifluorescence microscope. If the size of a fluorescent nanoparticle is beneath the Rayleigh resolution of the microscope, then the image of the nanoparticle will appear as the point spread function of the microscope, which is well-approximated by a Gaussian function. The center position of the point spread function is calculated by nonlinear regression to a Gaussian. Translations and rotations of the MEMS device are measured by computing the rigid transform that best maps the calculated positions of the point sources in the constellation between each pair of consecutive images.

The standard uncertainties of such measurements can be determined empirically. Estimates of the uncertainty achievable under ideal circumstances, known as the *localization precision*, can also be predicted theoretically. The difference between the experimentally tested measurement uncertainty and the theoretically predicted localization precision provides an assessment of the errors due to non-idealities in the experimental setup and indicates the improvement achievable from refinement of the measurement technique.

Research Aim

Many mechanical systems can be described as rigid bodies related by boundary conditions. The motion of such systems is specified by a set containing 3 points for each rigid body (two points per body for planar systems). Measurement of the point trajectories is equivalent to determining the system kinematics. As an industrially-important example, microfabricated accelerometers and gyroscopes could be well characterized by rigid body kinematics if empirical measurement techniques were available. Such techniques must be able to capture operating frequencies from 1 Hz to 1 MHz with characteristic displacements ranging from 10 nm to 10 μm. Wide-field super-resolution fluorescence microscopy poses a promising solution capable of exceptionally high accuracy.

Conclusions

The experimental uncertainty of the displacement measurements presented here was found to exceed the theoretical value by an order of magnitude. This indicates that the limiting factors in the uncertainty of the measurements described here are not accounted for in the idealized localization precision that is commonly used to express the minimum achievable uncertainty of super-resolution fluorescence microscopy measurements. Reductions in microscope stage drift and the addition of stationary reference nanoparticles in the field of view of the microscope together provide a clear path towards an improved experimental measurement uncertainty of MEMS kinematics.

Fluorescence Microscopy of Mechanical Systems

In a wide-field imaging system, a light source that is smaller than the imaging wavelength appears as the system's point spread function (PSF). When a contrast enhancement technique such as fluorescence microscopy is employed, super-resolution image analysis of the PSF can be used to localize such a point source with precision many orders of magnitude smaller than the imaging wavelength. If the point source is rigidly fixed to a component of a mechanical system, displacements of the component can be inferred from translations of the observed point spread function.

Colloidal suspensions of fluorescent nanoparticles having diameters less than half of their emitted wavelength can be obtained from commercial sources. These nanoparticles can be rigidly fixed to mechanical components by diluting the suspension and depositing a droplet with a micropipette. Evaporation of the liquid leaves behind the nanoparticles, adhered to the surface of the component.

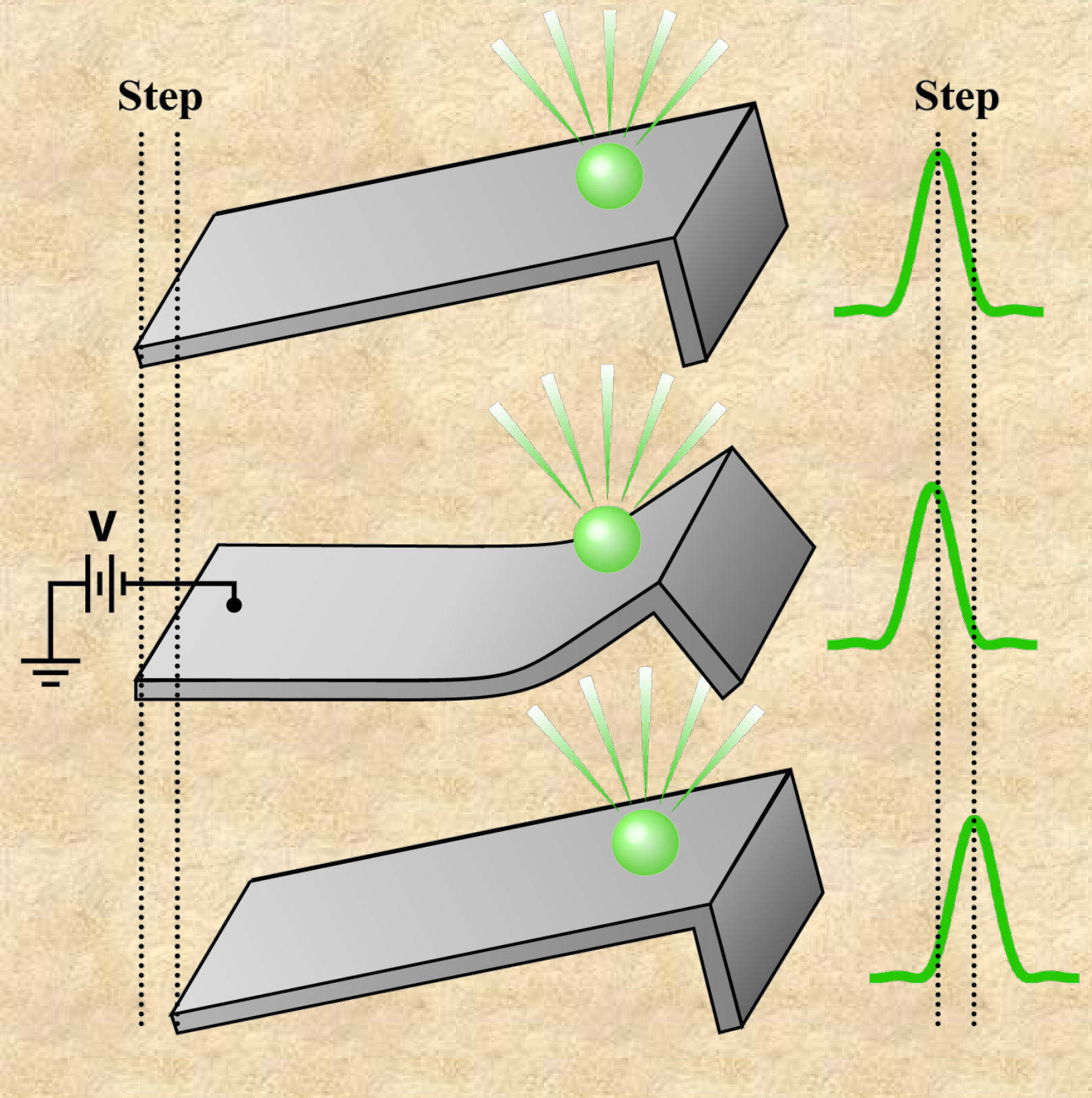

Figure 1: Super-resolution fluorescence microscopy of a MEMS actuator. Voltage cycling moves the actuator forward in a stepwise manner. Fluorescent nanoparticles deposited onto the actuator appear as point sources of light in fluorescence micrographs, creating point spread patterns in the collected images. Fitting these patterns by non-linear regression allows measurement of the actuator displacement with 1.85 nm uncertainty.

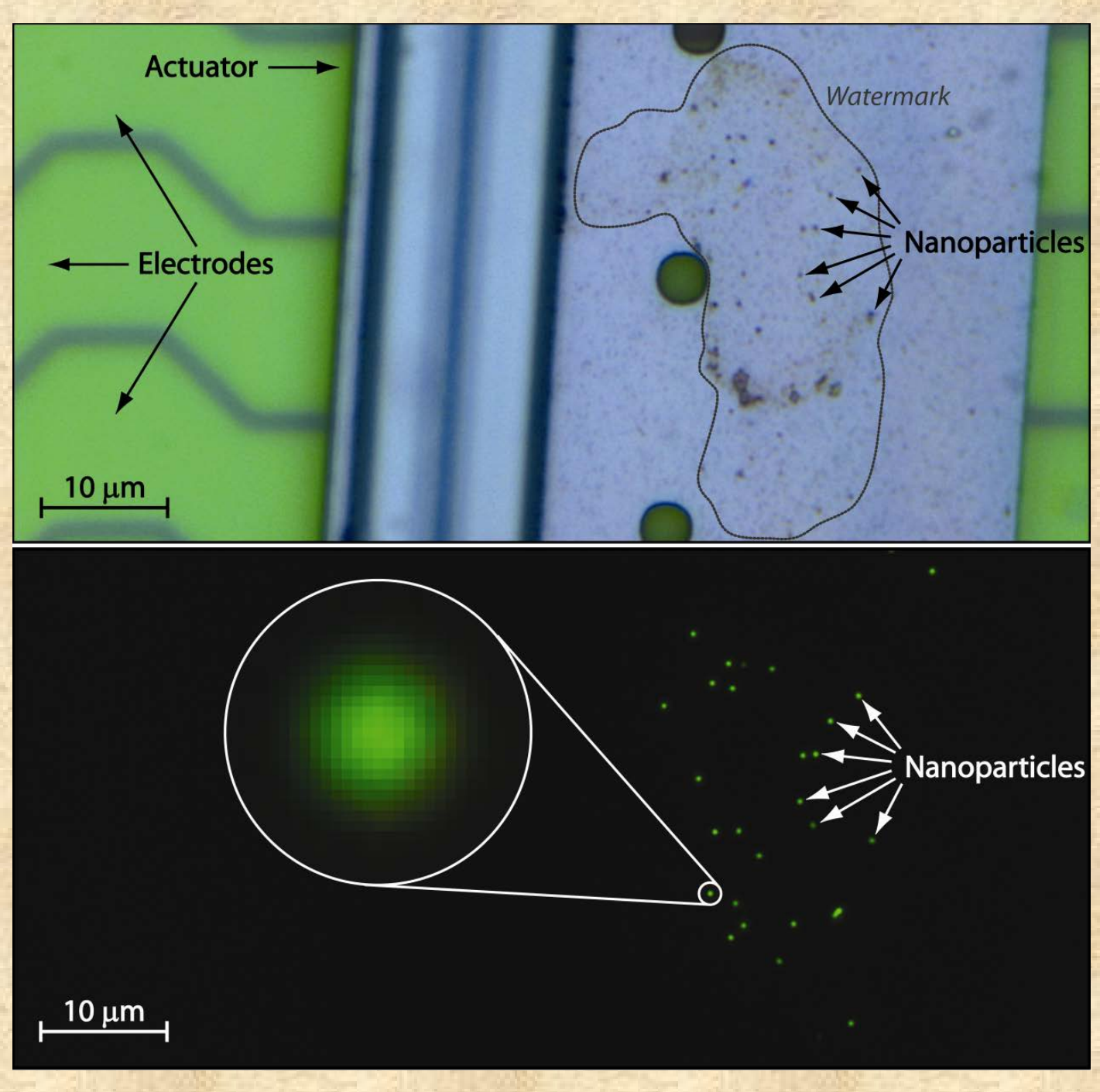

Figure 2: A constellation of fluorescent nanoparticles on a MEMS actuator. A brightfield micrograph (**top**) shows the structure of the actuator. The nanoparticles are barely visible. A fluorescence micrograph (**bottom**) shows the nanoparticles with high contrast.

Point-Source Constellation Tracking

A constellation consisting of many fluorescent nanoparticles can provide much more information than can be obtained by tracking a single nanoparticle only. The use of constellations allows tracking rotation and deformation of a mechanical system and reduces the attainable measurement uncertainty.

L_c

$$L_c = \sqrt{\frac{16\left(\sigma^2 + a^2/12\right)}{9N\eta} + \frac{8\pi b^2\left(\sigma^2 + a^2/12\right)}{a^2 N^2 \eta}}$$

$L_c =$ Localization Precision
$\sigma =$ Std. Dev. of PSF
$a =$ Sampling Pitch of CCD
$b^2 =$ Background Photons per Pixel
$N =$ Number of Photons per Point
$\eta =$ Number of Points in Constellation

Theoretical Limits

The ***localization precision*** of a fluorescence microscopy measurement reflects the displacement uncertainty that is theoretically achievable with super-resolution analysis, given certain assumptions about the instrumentation. Under the idealized model used to calculate the localization precision, the uncertainty is limited by the total number of detected photons that form the image of each fluorescent nanoparticle, the number of detected background photons per pixel, the point spread function of the microscope, the magnified sampling pitch of the CCD detector, and the number of nanoparticles in the constellation.

The optical system used for the experiment described in this poster had a fitted Gaussian standard deviation of $\sigma = 160$ nm and a green channel sampling pitch of $a = 46.82$ nm after accounting for the Bayer filter pattern of the color CCD camera. During a 400 ms exposure time, an average of $N = 257{,}000$ photons were detected from each of $\eta = 10$ nanoparticles in the constellation used for the experiment. The measured average background intensity was $b^2 = 15$ detected photons per pixel. The localization precision of an individual nanoparticle was therefore determined to be $L_P = 0.42$ nm, and the localization precision of the constellation was found to be $L_C = 0.13$ nm.

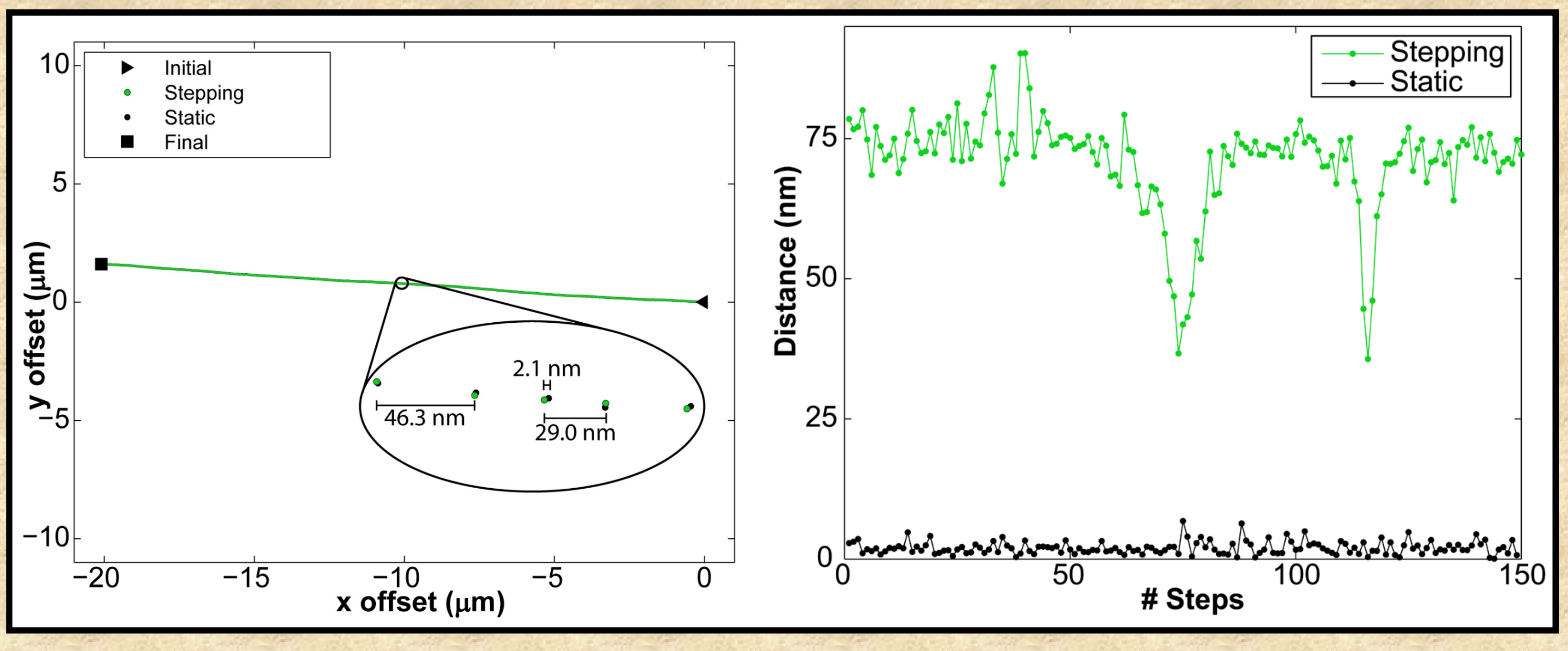

Figure 3: Trajectory (**left**) and time-series step size data (**right**) of a MEMS actuator as measured by point source constellation tracking. Data points shown in green represent actuator steps, whereas black data points represent the measured position or distance during an interval when the actuator was not stepping. These latter measurements indicate the uncertainty of the measurement technique.

Experimental Uncertainties

Experimental tests were conducted on a MEMS scratch drive actuator labeled with a constellation of 10 fluorescent polystyrene nanoparticles, each having a diameter of approximately 200 nm. The actuator was operated for a single duty cycle and then stopped. While the actuator was immobile, two sequential fluorescence micrographs were captured on a charge-coupled device (CCD) digital camera and were stored for subsequent analysis. This procedure of a single actuator step followed by two images was repeated 150 times for a total of 300 images. The position of each nanoparticle within the orthogonal (x, y) coordinate frame of the image was determined by Gaussian estimation, and the centroid of the nanoparticle constellation in each image was calculated. Each pair of sequential images that were separated in time by an actuator step provided a measurement of the actuator step size. Each pair of sequential images that were not separated in time by a step of the actuator provided a sample of the measurement noise. Time series data on the actuator step size and the empirical measurement noise are plotted in Figure 3.

The pair of images following each actuator step provides two independent samples from the population of possible observed positions for each coordinate of the centroid of the nanoparticle constellation. The mean and standard deviation can be extracted from the sample pair. From the set of 150 collected sample pairs, a pooled standard deviation of the measurement can be extracted for the x coordinate and another for the y coordinate. These pooled standard deviations describe statistical x-axis and y-axis uncertainties of the centroid position measurement. The standard uncertainty of the centroid displacement measurement is therefore the sum in quadrature of uncertainties from four independent measurements: two x-axis position measurements and two y-axis position measurements. The standard uncertainty of the x-axis position was found to be 0.91 nm, while the standard uncertainty of the y-axis position was 0.94 nm. The combined standard uncertainty of the displacement measurement was therefore 1.85 nm. Similarly, a pooled standard deviation of the orientation of the constellation can be used to calculate the standard uncertainty of the stepwise rotation. The standard uncertainty of orientation was found to be 70.7 μrad, and the combined standard uncertainty of the stepwise rotation measurement was 100 μrad.