3D particle tracking for microscale characterization of polymer networks

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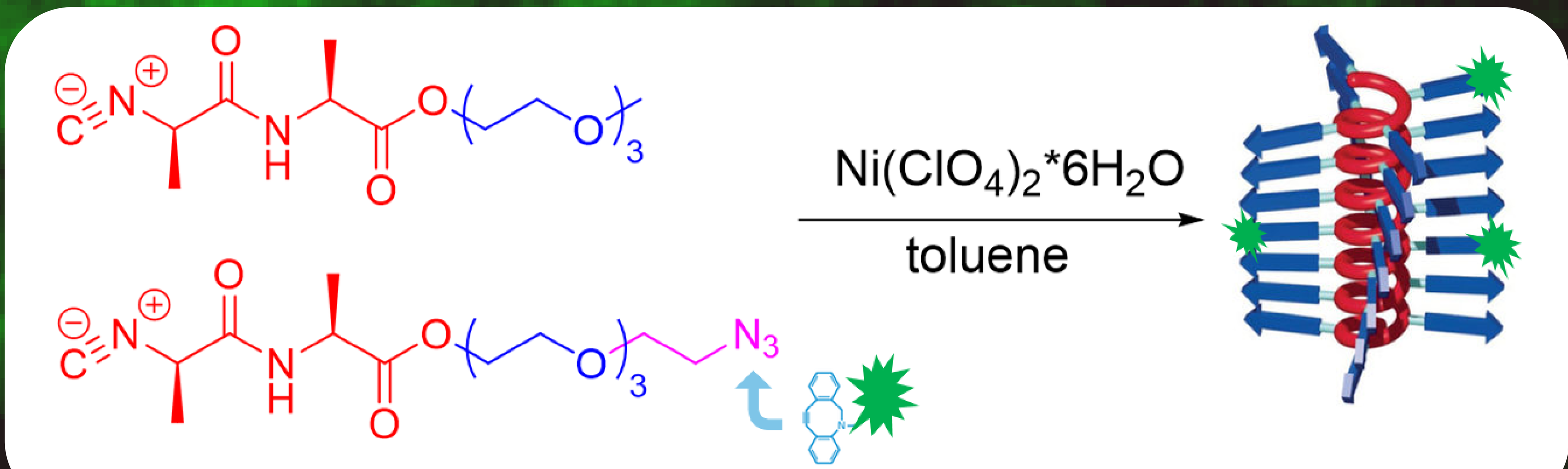
Introduction

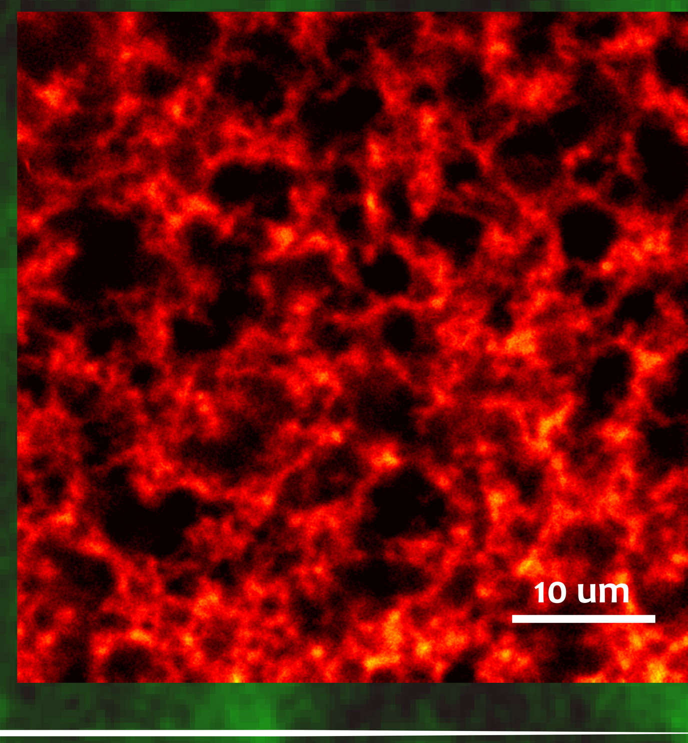
Polyisocyanopeptide (PIC) hydrogels are a new generation of synthetic material that possess visco-elastic properties close to biopolymer networks, such as collagen. One of their main advantages over their non-synthetic counterpart is that their mechanical properties can be easily tuned. Despite being well characterized at the macroscale using bulk rheology, their properties at the microscale remain, to a large extent, unknown.



Kouwer, P. H. J. . Responsive biomimetic networks from polyisocyanopeptide hydrogels. Nature 493, 651–655 (2013).

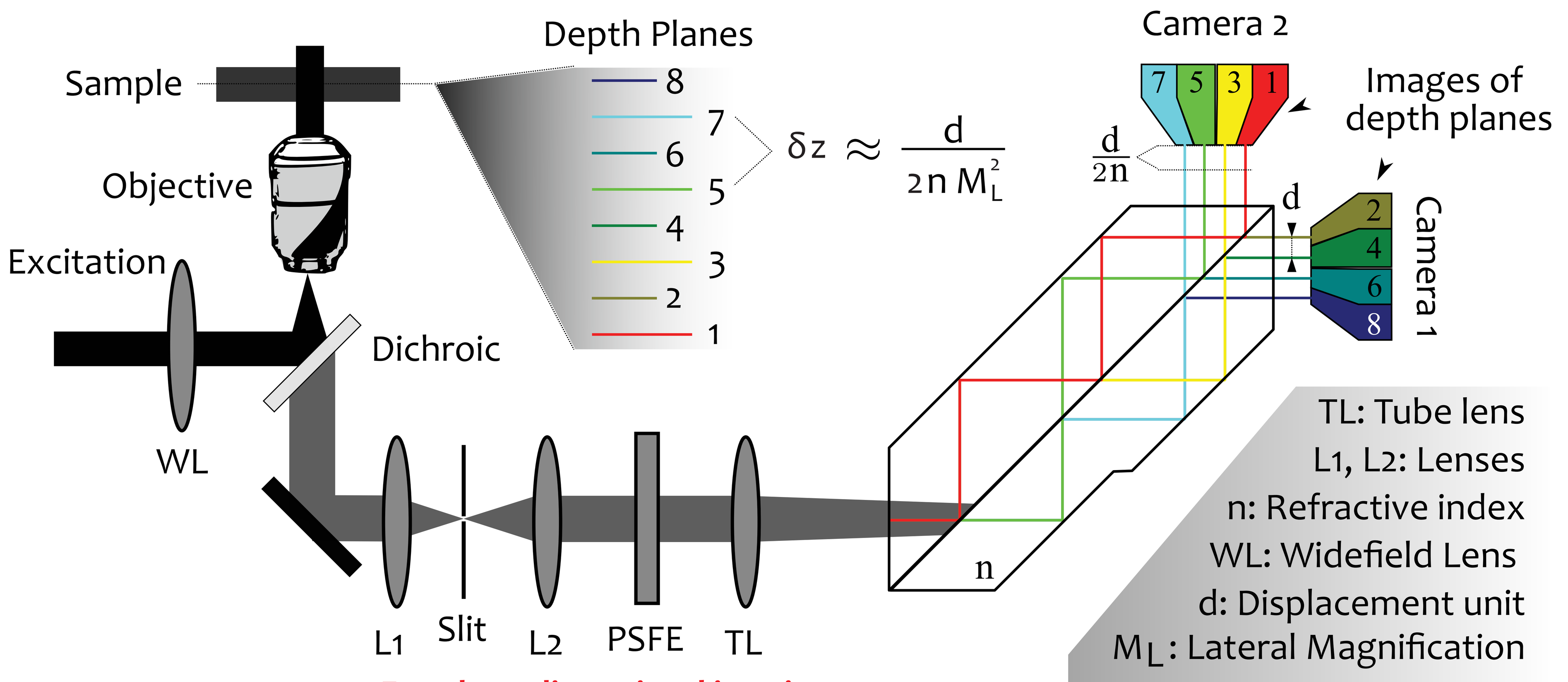
Polyiscyanopeptide (PIC) is used here as a model polymer to develop a data acquisition framework and analysis software for broader applications.

PIC network - confocal

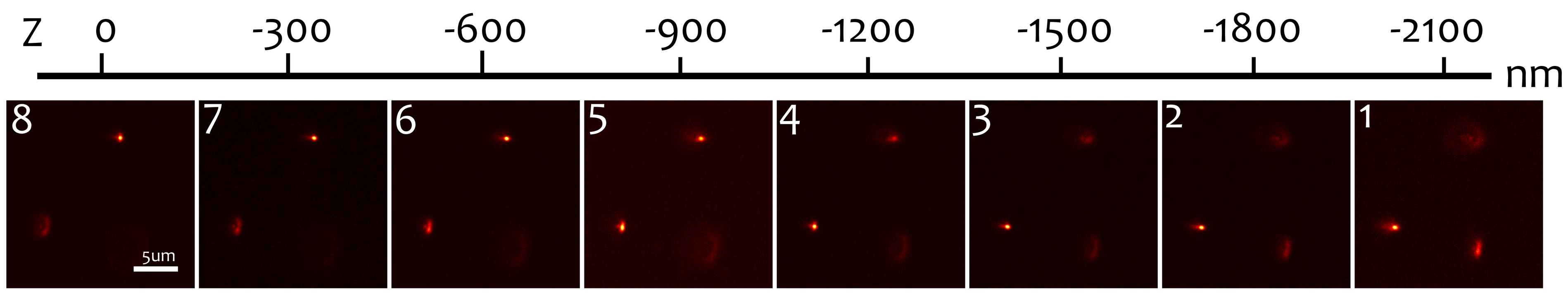


Methods

Multiplane widefield Imaging

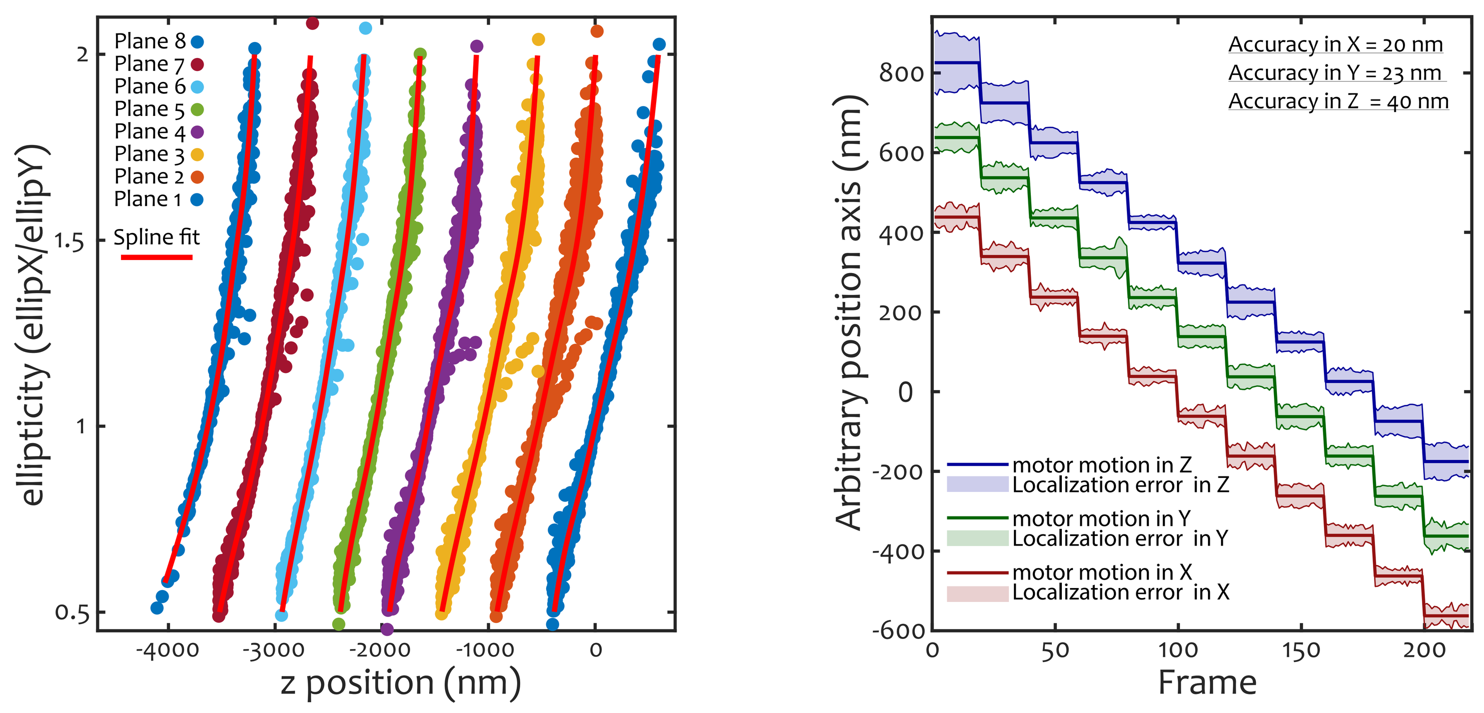


Fast three-dimensional imaging

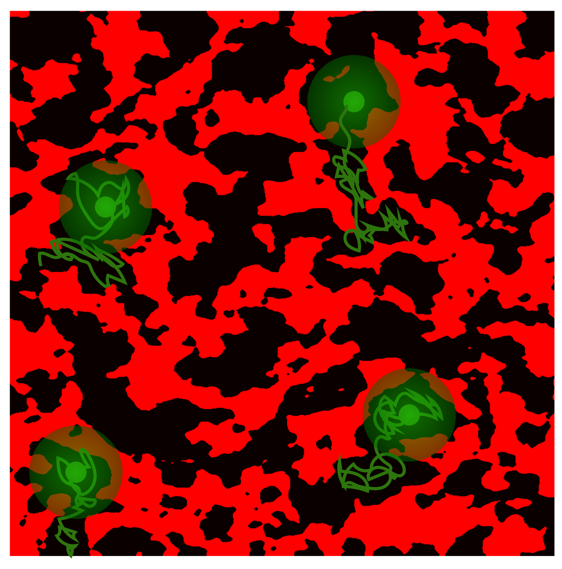


8 different focal planes can be acquired simultaneously

XYZ - Calibration



Toward micro-rheology

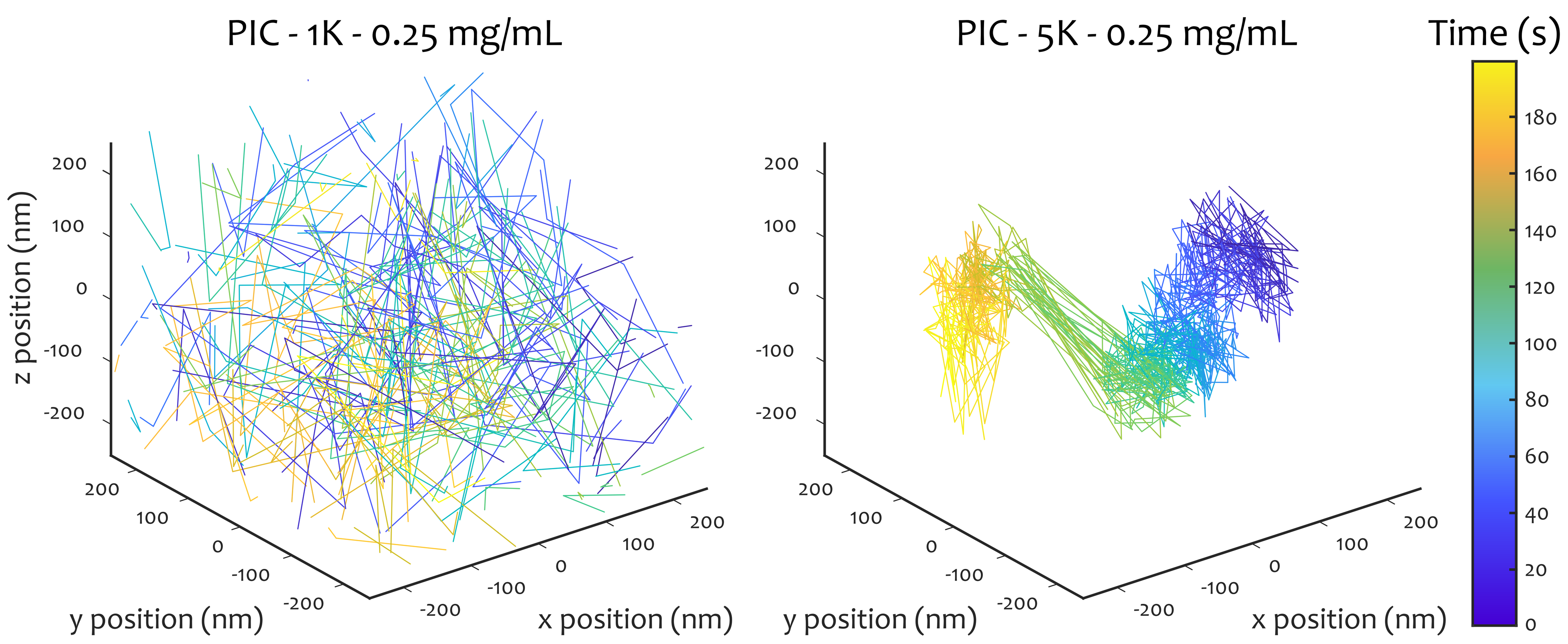


Work-flow:

1) Measure fluorescent marker embedded in the material of interest (Here PIC gel)
2) Localization of the markers on each plane from each frame
3) Connecting localizations belonging to the same molecules across the different planes
4) Connecting particles along frames (= tracking)
5) Extracting distances and mean squared dis-placement

Results

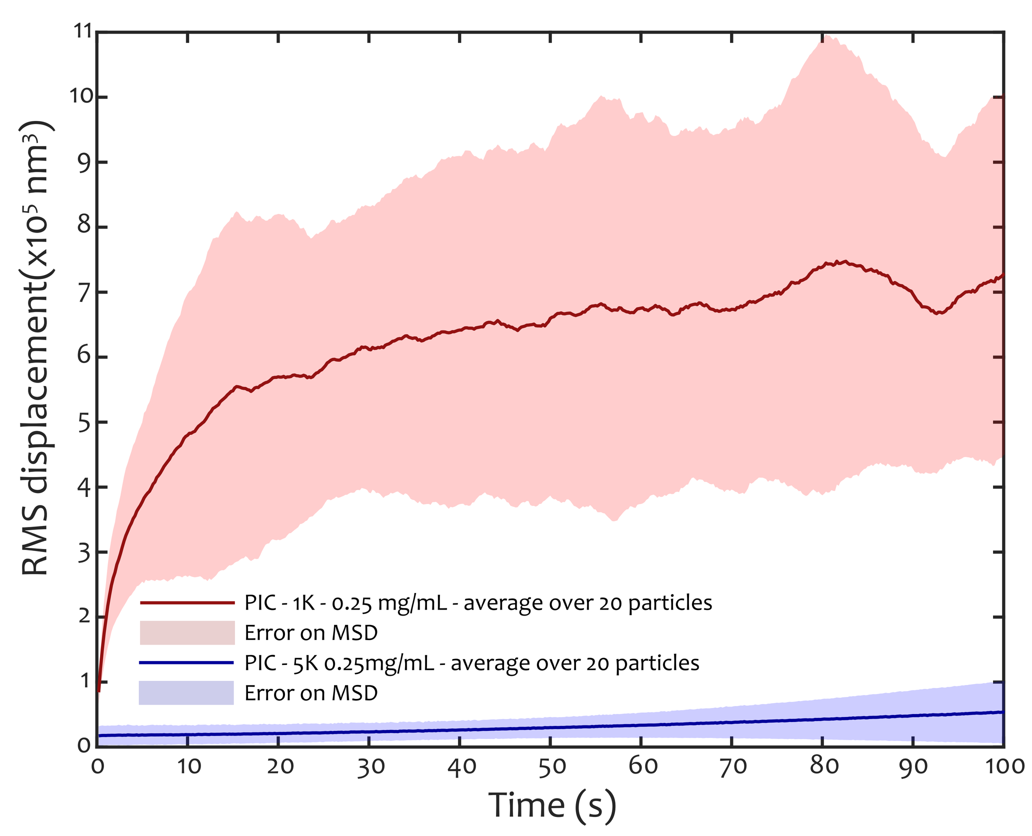
3D single-Particle tracking (Fluorescent beads in PIC gel)



Observation:
- The diffusion volume is much larger for shorter polymer lenght (1K) than for longer ones (5K)
- The motion is also much more random for 1K polymer than for 5K as brought to light by the time-color-coding

These observations are in good agreement with the bulk rheology of the material for 1K than 5K at low concentration (e.g. 0.25 mg/mL) showing the applicability of micro-rheology to investigate this material.

Mean square displacement



Observation:
- Confirming the previous observation we see that the beads have a higher diffusion coefficient in 1K polymer gel.
- We also see that despite the larger motion, the movement of the particle is confine.

Acknowledgement

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Conclusions & Outlook

The 3D tracking based on multi-plane imaging presented here showed high accuracy in all three dimensions. Additionally, we showed that we could accurately follow the motion of fluorescent beads embedded in a gel (here Polyisocyanopeptide) matrix in a large volume (> 50 x 50 x 5 μm^3). Moreover, we showed that the motion of the beads was dependent on the length of the polymer used for the gel. These results are consistent with the bulk rheology of this material. It worth pointing out the large standard deviation in RMS. This is likely due to the inhomogeneity of the polymer which leads to each particle "feeling" a different environment and thus behave differently. This is the main motivation of using such approach to characterize polyisocyanopeptide gels as it provide micro-scale information about the gel. Further investigation will be conducted as well as two-point rheology to fully characterize the polymer.