Thermophoretic Trap for Single Amyloid Fibril and Protein Aggregation Studies

Frank Cichos (✉ cichos@physik.uni-leipzig.de)

Peter Debye Institute for Soft Matter Physics, Molecular Nanophotonics Group, Leipzig University,
Linnéstr. 5, 04103 Leipzig, Germany

Martin Fränzl

Peter Debye Institute for Soft Matter Physics, Molecular Nanophotonics Group, Leipzig University,
Linnéstr. 5, 04103 Leipzig, Germany

Tobias Thalheim

Peter Debye Institute for Soft Matter Physics, Molecular Nanophotonics Group, Leipzig University,
Linnéstr. 5, 04103 Leipzig, Germany

Juliane Adler

Institute for Medical Physics and Biophysics, Leipzig University, Härtelstr. 16-18, 04107 Leipzig,
Germany

Daniel Huster

Institute for Medical Physics and Biophysics, Leipzig University, Härtelstr. 16-18, 04107 Leipzig,
Germany

Juliane Posseckardt

Kurt-Schwabe-Institut für Mess- und Sensortechnik e.V. Meinsberg, 04736 Waldheim, Germany

Michael Mertig

Kurt-Schwabe-Institut für Mess- und Sensortechnik e.V. Meinsberg, 04736 Waldheim, Germany

Method Article

Keywords: Plasmonics, Trapping, Brownian Motion, Thermophoresis, Amyloid Fibrils, Protein Aggregation

Posted Date: March 22nd, 2019

DOI: <https://doi.org/10.1038/protex.2019.031>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

The observation of single freely suspended amyloid fibrils in liquids for minutes and hours is due to Brownian motion very difficult. We present a force-free trapping method in this protocol, which is able to confine the motion of these objects to a region of interest. To achieve this confinement, we use thermophoretic drifts of molecules suspended in a temperature gradient in liquids. The temperature gradient is generated by a metallic nano-structure that is heated by a focused laser beam. The amyloid fibrils are trapped in the temperature minimum created above the structure since most molecules and nano-objects move towards the cold. The trapping capability is determined by the ratio of the thermophoretic mobility to the diffusion coefficient, which is called Soret coefficient, and the temperature contrast in the liquid.

Introduction

The tracking of the dynamics of single molecules and nano-objects over time has attracted growing interest^{1,2}. One major example where this can be applied is the process of nucleation and growth of macromolecular aggregates. Protein aggregation into amyloid fibrils yields structures associated to physiological functions in organisms whereas a multitude of other amyloids are connected to diseases³⁻⁸. Nevertheless, common techniques relying on ensemble measurements lack the possibility to follow details on nucleation of oligomers, fibril growth, fragmentation, and secondary nucleation as all these processes occur at the same time. The ensemble average blurs these fundamental single steps, thus much effort in sophisticated modeling^{9,10} or other well-designed experiments is required^{11,12}. We present a trap in this protocol which works with the actual fuel of Brownian motion – temperature – to confine single amyloid fibrils to a region of interest over extended time periods of up to several hours. The fibrils are in solution during the experiments and no surface immobilization is necessary. Trapping is enabled by temperature gradients which lead to thermophoretic drifts of the investigated fibrils due to temperature-dependent solute-solvent interactions¹³⁻¹⁶. A drift velocity v_T which results from these interactions can be assigned to the temperature gradient ∇T via $v_T = -D_T \nabla T$. D_T is called thermodiffusion coefficient and describes the strength of the thermophoretic drift. Different physical mechanisms contribute to this coefficient¹⁴, however, in most cases D_T is found to be positive, i.e., the corresponding object moves from the hot to the colder region in the temperature gradient. A measure for the relative strength of these drifts can be found in the Soret coefficient S_T defined as the ratio of the thermodiffusion coefficient over the diffusion coefficient: $S_T = D_T / D$. Typical values range in between $S_T = 0.01 \dots 10 \text{ K}^{-1}$ depending on the size and the composition of the object of study. We generate temperature fields by optically heating one chrome ring out of a whole array deposited on a glass cover slip utilizing a focused laser beam (beam waist $\omega_0 \approx 500 \text{ nm}$) of 808 nm wavelength to provide high temperature gradients at low temperature increments (see Figure 1). By rotating the focused laser beam at a frequency of $f = 100 \text{ Hz}$ on the chrome ring with the help of an acousto-optic deflector, a time averaged temperature field can be generated which has its minimum in the center and is

maximal at the chrome structure. Objects with a positive Soret coefficient are thus confined to the center of the trap. The thickness of the sample is controlled with a second cover slip.

Figure 1

Fig. 1: Sketch of the trapping mechanism. **A** static temperature field is generated by a chrome ring which is heated by a focused laser beam rotating at a high frequency on the ring.

This protocol describes the setup for such single-fibril trapping experiments and provides a step-by-step manual for the production of the chrome structure using UV lithography, the preparation of A β ₄₀, A β ₄₂ and pGlu₃-A β ₃₋₄₀¹⁷ amyloid fibrils as example systems, and setting up a measurement sample. All optical components are selected according to the fluorescent staining of the fibrils with Thioflavin T^{18,19}. The configuration of lasers (and therefore the acousto-optic deflector, the dichroic mirror and the filters which are designed for a specific wavelength or wavelength regime) might change for systems labeled with other dyes. Contrary to feedback systems^{20,21}, trapping of an object in our thermophoretic setup does not require continuous fluorescence excitation due to the static temperature landscape. Hence, experiments with periods in which the laser for excitation is switched off (intermittent observation) drastically reduce photo-bleaching effects and permit long-time studies up to several hours. Nevertheless, combined with a real time tracking routine a feedback system is available as well²². In the presented case of a nearly harmonic temperature profile with a curvature α (see²²), all objects with a Soret coefficient S_T can be trapped for which the radius of the metal ring is larger than the width of the resulting position distribution $\sigma = 1 / (-S_T \alpha)^{0.5}$. If no UV lithography setup is accessible, an alternative method resulting in a random distribution of traps on a cover slip is polystyrene bead lithography described in²³.

Reagents

UV Lithography • Acetone • Isopropyl • Milli-Q water • Adhesion promoter (TI PRIME Adhesion Promoter, MicroChemicals) • Negative photoresist (AZ nLOF 2020, MicroChemicals) • Developer (AZ 326 MIF, MicroChemicals) • Remover (TechniStrip NI555, MicroChemicals) **Fibril Preparation** • NaCl (Carl Roth) • TRIS (C₄H₁₁NO₃, Carl Roth) • Na₂HPO₄ (Carl Roth) • NaH₂PO₄ (Carl Roth) • NaOH (Carl Roth) • HCl (Carl Roth) • Milli-Q water • Lyophilized A β ₄₀ (Core Unit Peptide, Leipzig University) • Lyophilized A β ₄₂ (Core Unit Peptide, Leipzig University) • Lyophilized pGlu₃-A β ₃₋₄₀ (Core Unit Peptide, Leipzig University) **Sample Preparation** • Acetone • Isopropyl • Toluene (Carl Roth) • Milli-Q water • Pluronic F-127 (Sigma Aldrich) • Polystyrene (PSS Polymer, $M_w = 88$ kDa, PDI = 1.66) • Poly(dimethylsiloxane) (PDMS, Sigma Aldrich) • Thioflavin T (Sigma Aldrich)

Equipment

Microscopy Setup • Computer with LabVIEW • Inverted microscope (IX73, Olympus) • Objective lens (Olympus 100x/1.3 oil) • 445 nm CW laser (MDL-III-445-200, CNI) • 808 nm CW laser (PL.MI.808.300,

Pegasus Lasersysteme) • Piezo translation stage \ (P-733.3, Physik Instrumente) • Acousto-optic deflector \ (2DS-75-40-808, Brimrose) • Dichroic beam splitter \ (XF2034, Omega Optical) • EMCCD \ (Andor iXon 3) • AD/DA converter \ (ADwin-Gold II, Jäger Messtechnik) • Bandpass filter \ (ET490/40, Chroma) • Short-pass filter \ (FESH0750, Thorlabs) • 500 mm tube lens \ (Thorlabs) • Pellicle beam splitter \ (Thorlabs) • Standard optical components as mirrors and lenses \ (Thorlabs) ****UV Litographpy**** • AutoCAD file with designed chrome structure • Photolithography room \ (no light components below 500 nm for ambient illumination) • Exhaustive hood \ (Prutscher Laboratory Systems) • 22 mm x 22 mm glass cover slips \ (H874.2, Carl Roth) • Hotplate \ (MR3003 control, Heidolph Instruments) • Spin coater \ (SÜSS MICROTECH) • UV lithography system \ (MJB4 Mask-Aligner, Süss MicroTech) • Photomask \ (3" photomask, Rose Fotomasken) • Thermal evaporation system \ (UNIVEX 300, Leybold) • Ultrasonic bath \ (SONOREX, Bandelin GmbH) ****Fibril Preparation**** • ThermoMixer \ (Eppendorf) • pH meter \ (Lab 850, Schott Instruments) • Dialysis tube \ (Spectra/Por) • Standard biochemical lab equipment ****Sample Preparation**** • Exhaustive hood \ (Prutscher Laboratory Systems) • Storage vial \ (XC37.1, Carl Roth), Caps for storage vial \ (XC44.1, Carl Roth) • PARAFILM M \ (Sigma Aldrich) • 22 mm x 22 mm glass cover slips \ (H874.2, Carl Roth) • Microcentrifuge tubes black \ (AA80.1, Carl Roth) • Nitrogen gun • Spin coater \ (WS-650S-6NPP/Lite Spin Processor with 650 Controller, Laurell Technology Corporation) • Cannula \ (X133.1, Carl Roth) • Sample holder for microscopy \ (Homemade) • Standard biochemical lab equipment

Procedure

****Microscopy Setup**** A sketch of the experimental setup can be found in Figure 2. The setup is built around an inverted microscope. A piezo translation stage mounted to the microscope is used for fine-positioning of the trap right before a trapping experiment starts. The beam diameter of a CW laser at a wavelength of 808 nm used for the heating of the chrome ring is increased by a beam expander. Afterwards, the widened laser beam is sent through an acousto-optic deflector \ (AOD) and a lens system which allows for the steering of the laser focus in the sample plane on the circumference of the chrome ring. Fluorescence excitation of the labeled fibrils is done with a 445 nm CW laser which is focused to the back-focal plane of the objective to attain a homogeneous illumination of the sample in the trapping region. Both laser beams are merged by a pellicle beam splitter \ (BS). A dichroic mirror \ (D) is selected according to the excitation and the heating laser wavelengths as well as the emission wavelength of the labeled sample. Reflections of the heating laser in the detection pathway are blocked with a short-pass filter. Fluorescent light of the sample is imaged to an EMCCD after passing a bandpass filter \ (F) as well as a tube lens. The AD/DA converter drives the acousto-optic deflector and the piezo stage. The fluorescence is imaged in real time and the control of the AD/DA converter \ (ADwin) is done with a custom LabVIEW program.

Figure 2

****Fig. 2: Sketch of the experimental setup. ****

****UV Lithography**** UV lithography is used to provide glass cover slips with the chrome structures. This chrome structure constitutes the traps for the thermophoretic experiments. As the photoresist used for the

UV lithography is highly sensitive to visible light in the lower spectral range and UV light, the procedure has to be executed in a specific photolithography room. Commonly, the ambient illumination in these rooms is realized with yellow fluorescent light tubes containing filters blocking all light components which are below 500 nm. A photomask for the UV lithography system was produced according to an AutoCAD file with the designed chrome structures as shown in Figure 3.

Figure 3

***Fig. 3: CAD images of the chrome structure.** The chrome traps are arranged in arrays. On each quadrant of the cover slip arrays of rings can be found with inner diameters of 8 μm , 10 μm , 14 μm , and 20 μm . They are separated by a cross. The capital "L" in the left top corner indicates the upper side on the cover slip.*

1. Thoroughly clean a glass cover slip with acetone and isopropyle.
2. Dry the clean glass cover slip on the hotplate at 120 °C for 10 min.
3. During the previous step, switch on the UV lithography system and install the photomask.
4. Fix the cleaned glass cover slip on the spin coater.
5. Spin coat 100 μl of the adhesion promoter on the glass cover slip at 3000 rpm for 30 s using dynamic dispense spin coating technique. No residual drops or film of the adhesion promoter should be visible after spin coating.
6. Bake the adhesion promoter immediately after spin coating at 120 °C on the hotplate for 2 min.
7. Fix the cover slip on the spin coater.
8. Spin coat 200 μl of the negative photoresist on the glass cover slip at 3000 rpm for 30 s using dynamic dispense spin coating technique.
9. Soft-bake the photoresist immediately after spin coating at 110 °C on the hotplate for 60 s.
10. Fix the cover slip to the UV lithography system and align it with the photomask (for optimal exposure).
11. Expose the cover slip to the UV laser (66 mJ cm^{-2} , i-line).
12. Dismantle the cover slip from the UV lithography system and bake it at 110 °C for 60 s.
13. Develop the photoresist for 60 s. For this purpose, fill a 50 ml baker with the developer and wave the cover slip for exactly 60 s in the baker with a tweezer under the exhaustive hood.
14. Thoroughly rinse the cover slip with Milli-Q water. The future chrome structures should already be visible on the cover slip at this point.
15. Thermally evaporate a 30 nm chrome layer on the cover slip.
16. Strip the resist with the remover in the ultrasonic bath for 30 min. The chrome structure remains.
17. Thoroughly rinse the cover slip with Milli-Q water and dry it with nitrogen.

****Fibril Preparation****

*****a) Preparation of buffer solutions****

Preparation of phosphate buffer (pH 8.8 and pH 9.2):

1. Dilute a 500 mM Na_2HPO_4 stock solution with Milli-Q water to a final concentration of 25 mM.
2. Add the respective amount of NaCl for salt dependent measurements.
3. Adjust the pH value with NaOH.
4. Store the buffer solution at room temperature.

Preparation of phosphate buffer (pH 7.4):

1. Mix 500 mM stock solutions of Na_2HPO_4 and NaH_2PO_4 at a ratio of 8.1:1.9.
2. Dilute the solution with Milli-Q water to a final concentration of 25 mM.
3. Add the respective amount of NaCl for salt dependent measurements.
4. Adjust the pH value with NaOH or H_3PO_4 , respectively.
5. Store the buffer solution at room temperature.

Preparation of phosphate buffer (pH 6.1):

1. Mix 500 mM stock solutions of Na_2HPO_4 and NaH_2PO_4 at a ratio of 1.5:8.5.
2. Dilute the solution with Milli-Q water to a final concentration of 25 mM.
3. Add the respective amount of NaCl for salt dependent measurements.
4. Adjust the pH value with NaOH or H_3PO_4 , respectively.
5. Store the buffer solution at room temperature.

Preparation of TRIS buffer (pH 8.0):

1. Dissolve TRIS in Milli-Q water to a

concentration of 50 mM. 2. Add the respective amount of NaCl for a 100 mM concentration in the buffer. 3. Adjust the pH value with HCl. 4. Store the buffer solution at room temperature. ****b) Preparation of fibrils**** Preparation of A β ₄₀ fibrils: 1. Dissolve the lyophilized A β ₄₀ peptide in 25 mM phosphate buffer (pH 8.8) containing 150 mM NaCl to a concentration of 1 mg ml⁻¹. 2. Incubate the solution at 37 °C while shaking at 450 rpm for at least 12 h. 3. Store the fibril stock solution at 4 °C. Preparation of A β ₄₂ fibrils: 1. Dissolve the lyophilized A β ₄₂ peptide in 25 mM phosphate buffer (pH 9.2) containing 150 mM NaCl to a concentration of 0.25 mg ml⁻¹. 2. Incubate the solution at 37 °C while shaking at 450 rpm for 4 days. 3. Store the fibril stock solution at 4 °C. Preparation of pGlu₃-A β ₃₋₄₀ fibrils: 1. Dissolve the lyophilized peptide in 50 mM TRIS buffer (pH 8.0) containing 100 mM NaCl to a concentration of 0.1 mg ml⁻¹. 2. Incubate the solution at 37 °C while shaking at 450 rpm for 4 days. 3. Store the fibril stock solution at 4 °C. Preparation for salt and pH value dependent measurements: The fibril stock solution was dialyzed against the desired buffer solution using a dialysis tube with molecular weight cut-off (MWCO) of 1000. 1. Pipette 1 ml of the fibril solution into the dialysis tube and seal it with a clamp. 2. Fill 400 ml of the desired buffer into a beaker. 3. On stirring, dialyze the fibril solution against the desired buffer for 2 h. 4. Exchange the desired buffer solution in the beaker with a fresh one and dialyze again the fibril solution for 2 h. 5. Pipette the fibril solution into a microcentrifuge tube and store it at 4 °C. ****Sample Preparation**** ****a) Groundwork**** Preparation of a 1 mM ThT stock solution: 1. Weigh the corresponding amount of ThT powder for a 1 mM ThT solution. 2. Mix the ThT powder with water in a black microcentrifuge tube. 3. Store the stock solution at 4 °C. Preparation of a 2 % polystyrene solution as hydrophobic layer: A closed polystyrene layer is spin-coated on the cover slips to obtain a defined hydrophobic layer for an optimal adsorption of the blocking agent Pluronic F-127. 1. Weigh the corresponding amount of polystyrene for a 2 % solution. 2. Dissolve the polystyrene resin with toluene under an exhaustive hood. Take a storage vial with a toluene resistant cap. 3. Seal the cap with PARAFILM M for long-time storage at room temperature. Preparation of a 1 % Pluronic F-127 solution as anti-adsorbent: The Pluronic F-127 is always prepared freshly or at least once per week. 1. Mix Pluronic F-127 powder with Milli-Q water in a glass beaker to attain a 1 % Pluronic F-127 solution. 2. Cover the beaker with PARAFILM M to protect the solution from pollution. 3. Leave the beaker in an ultrasonic bath for 30 min for proper dissolving. 4. Store the solution at room temperature. ****b) Preparation of measurement sample**** The fibril solution is confined between two cover slips. One of the cover slips is prepared with the chrome structure for trapping. 1. Thoroughly rinse the cover slips with acetone, isopropyle and Milli-Q water. 2. Dry the clean cover slips with a nitrogen gun. 3. Spin coat 30 μ l of the 2 % polystyrene solution on the cover slips at 8000 rpm using dynamic dispense spin coating technique. The cover slip has to face the polystyrene layer with the chrome structure. 4. Immerse the cover slips in the 1 % Pluronic F-127 solution for 10 min. 5. Briefly dip the cover slips in Milli-Q water and dry them with nitrogen. Put them aside for the next few steps. 6. Prepare the fibril working solution by diluting 5 μ l of the fibril stock solution with 100 μ l Milli-Q water containing 0.1 % Pluronic F-127. (The amount of water is replaced with a corresponding amount of phosphate buffer or NaCl for pH and salt dependent experiments. 0.1 mg of the peptide monomers are added for growth experiments. The working solution commonly is prepared freshly right before a trapping experiment.) 7. Add 2 μ l of the 1 mM ThT stock solution to the working solution resulting in a ThT

concentration of about 20 μM . 8. Store the prepared working solution in darkness (e.g. using a black microcentrifuge vial or a film canister) at room temperature. 9. Cover the edges of the cover slip (from step 5) not containing the chrome structure with a thin PDMS layer for sealing using the cannula (cf. Figure 4). The polystyrene covered side of the cover slip is provided with PDMS here. 10. Pipette 0.35 μl of the final working solution in the middle of the cover slip containing the PDMS for sealing. (The amount of liquid used roughly defines the height of the sample during the experiments.) 11. Place the other cover slip containing the chrome structure on top with the chrome structure facing towards the solution. 12. Fix the sample on the microscopy sample holder with the cover slip containing the chrome structure on the top side and start the trapping experiment.

Figure 4

***Fig. 4: Procedure for setting up the measurement sample. ** The cover slip not containing the chrome structure is provided with PDMS at the edges. 0.35 μl of the sample solution is pipetted in the middle of the cover slip. Finally, the sample is sealed with the cover slip containing the chrome structure resulting in the completed measurement sample.*

Timing

****Microscopy Setup:**** Several weeks to months ****UV Lithography:**** $\approx 1\text{ h}$ ****Fibril Preparation:**** • Preparation of buffer solutions: $\approx 2\text{ h}$ • Preparation of fibrils: $\approx 12\text{ h}$ to 4 d • Preparation for salt and pH value dependent measurements: $\approx 5\text{ h}$ ****Sample Preparation:**** • Groundwork: $\approx 1\text{ h}$ • Preparation of measurement sample: $\approx 30\text{ min}$

Troubleshooting

Table 1

Anticipated Results

****UV lithography**** The quality of the produced traps can be assessed with a conventional microscope after the UV lithography procedure. Figure 5 shows an example of a successful preparation procedure recorded with a bright-field illumination microscope. The width of the chrome ring appears wider due to the limited optical resolution of the microscope.

Figure 5

Fig. 5: Example of produced trap for quality assessment.

****Sample preparation**** A successful preparation of the measurement sample results in freely diffusing fibrils not blurring when they are observed with the microscope described in the "Microscopy Setup" paragraph. Figure 6 shows two examples of camera snapshots.

Figure 6

Fig. 6: Snapshots of a successful preparation of a measurement sample showing Aβ40 fibrils in free diffusion (in the left image) and in free diffusion, but trapped (on the right image).

References

1. Manzo, C. & Garcia-Parajo, M. F. A Review of Progress in Single Particle Tracking: From Methods to Biophysical Insights. *_Rep. Prog. Phys._* 78, 124601 \ (2015).
2. Shen, H. et al. Single Particle Tracking: From Theory to Biophysical Applications. *_Chem. Rev._* 117, 7331–7376 \ (2017).
3. Dobson, C. M. Protein Folding and Misfolding. *_Nature_* 426, 884–890 \ (2003).
4. Chiti, F. & Dobson, C. M. Protein Misfolding, Functional Amyloid, and Human Disease. *_Annu. Rev. Biochem._* 75, 333–366 \ (2006).
5. Knowles, T. P. J., Vendruscolo, M. & Dobson, C. M. The Amyloid State and Its Association with Protein Misfolding Diseases. *_Nat. Rev. Mol. Cell Bio._* 15, 384–396 \ (2014).
6. Chiti, F. & Dobson, C. M. Protein Misfolding, Amyloid Formation, and Human Disease: A Summary of Progress Over the Last Decade. *_Ann. Rev. Biochem._* 86, 27–68 \ (2017).
7. Adamcik, J. & Mezzenga, R. Amyloid Polymorphism in the Protein Folding and Aggregation Energy Landscape. *_Angew. Chem. Int. Ed._* 57, 8370–8382 \ (2018).
8. Iadanza, M. G., Jackson, M. P., Hewitt, E. W., Ranson, N. A. & Radford, S. E. A New Era for Understanding Amyloid Structures and Disease. *_Nat. Rev. Mol. Cell Biol._* 19, 755–773 \ (2018).
9. Knowles, T. P. J. et al. An Analytical Solution to the Kinetics of Breakable Filament Assembly. *_Science_* 326, 1533–1537 \ (2009).
10. Michaels, T. C. T., Liu, L. X., Meisl, G. & Knowles, T. P. J. Physical Principles of Filamentous Protein Self-Assembly Kinetics. *_J. Phys.: Condens. Matter_* 29, 153002 \ (2017).
11. Knowles, T. P. J. et al. Observation of Spatial Propagation of Amyloid Assembly from Single Nuclei. *_Proc. Natl. Acad. Sci. USA_* 108, 14746–14751 \ (2011).
12. Linse, S. Monomer-Dependent Secondary Nucleation in Amyloid Formation. *_Biophys. Rev._* 9, 329–338 \ (2017).
13. Duhr, S. & Braun, D. Why Molecules Move along a Temperature Gradient. *_Proc. Natl. Acad. Sci. USA_* 103, 19678–19682 \ (2006).
14. Piazza, R & Parola, A. Thermophoresis in Colloidal Suspensions. *_J. Phys. Condens. Matter_* 20, 153102 \ (2008).
15. Würger, A. Thermal Non-Equilibrium Transport in Colloids. *_Rep. Prog. Phys._* 73, 126601 \ (2010).
16. Bregulla, A. P., Würger, A., Günther, K., Mertig, M. & Cichos, F. Thermo-Osmotic Flow in Thin Films. *_Phys. Rev. Lett._* 116, 188303 \ (2016).
17. Scheidt, H. A., Adler, J., Krueger, M. & Huster, D. Fibrils of Truncated Pyroglutamylo-Modified A β Peptide Exhibit a Similar Structure as Wildtype Mature A β Fibrils. *_Sci. Rep._* 6, 1–7 \ (2016).
18. Xue, C., Lin, T. Y., Chang, D. & Guo, Z. Thioflavin T as an Amyloid Dye: Fibril Quantification, Optimal Concentration and Effect on Aggregation. *_Royal Soc. Open Sci._* 4, 160696 \ (2017).
19. Shaban, H. A., Valades-Cruz, C. A., Savatier, J. & Brasselet, S. Polarized Super-Resolution Structural Imaging inside Amyloid Fibrils Using Thioflavine T. *_Sci. Rep._* 7, 12482 \ (2017).
20. Cohen, A. E. & Moerner, W. E. Suppressing Brownian Motion of Individual Biomolecules in Solution. *_Proc. Natl. Acad. Sci. USA_* 103, 4362–4365 \ (2006).
21. Jun, Y., Gavrilo, M. & Bechhoefer, J. High-Precision Test of Landauer’s Principle in a Feedback Trap. *_Phys. Rev. Lett._* 113, 190601 \ (2014).
22. Braun, M., Bregulla, A. P., Günther, K., Mertig, M. & Cichos, F. Single Molecules Trapped by Dynamic Inhomogeneous Temperature Fields. *_Nano Lett._* 15, 5499–5505 \ (2015).
23. Braun, M. & Cichos, F. Optically Controlled Thermophoretic Trapping of Single Nano-Objects. *_ACS Nano_* 7, 11200–11208 \ (2013).

Acknowledgements

F. C. and D. H. acknowledge financial support by the German Research Foundation DFG through the Collaborative Research Center TRR 102 "Polymers under multiple constraints: restricted and controlled molecular order and mobility", \(\backslash\)(Gefördert durch die Deutsche Forschungsgemeinschaft \(\backslash\)(DFG) – Projektnummer 189853844 – TRR 102) and project CI 33/14-1. J.P. and M.M. acknowledge financial support by the BMBF \(\backslash\)(Contract 03WKCL01G) and the DFG via the Cluster of Excellence "cfaed" \(\backslash\)(Contract EXC 1056/1).

Figures

Figure 1

Figure 2 Sketch of the experimental setup.

Figure 2

Figure 1 Sketch of the trapping mechanism. A static temperature field is generated by a chrome ring which is heated by a focused laser beam rotating at a high frequency on the ring.

Figure 3

CAD images of the chrome structure. The chrome traps are arranged in arrays. On each quadrant of the cover slip arrays of rings can be found with inner diameters of 8 μm, 10 μm, 14 μm, and 20 μm. They are separated by a cross. The capital "L" in the left top corner indicates the upper side on the cover slip.

Figure 4

Procedure for setting up the measurement sample. The cover slip not containing the chrome structure is provided with PDMS at the edges. 0.35 µl of the sample solution is pipetted in the middle of the cover slip. Finally, the sample is sealed with the cover slip containing the chrome structure resulting in the completed measurement sample.

Figure 5

Example of produced trap for quality assessment.

Figure 6

Snapshots of a successful preparation of a measurement sample showing Aβ~40~ fibrils in free diffusion (in the left image) and in free diffusion, but trapped (on the right image).

Step	Problem	Possible reason	Solution
UV Lithography			
Step 16	The chrome might detach at the rings as well.	The cover slip was not very clean.	More care for the cleaning process should be taken as sometimes pollutants stick firmly to the cover slide. Alternative, more elaborated cleaning methods incorporate a Hellmanex III (Z805939-1EA, Sigma Aldrich) or a Piranha etch (3:1 mixture of pure H ₂ SO ₄ and 35 % H ₂ O ₂) cleaning procedure and remove pollution more thoroughly.
Sample Preparation			
Step 11	The droplet of the sample does not spread appropriately over the sample.	Air bubbles are in the sample or the PDMS layer is too thick.	The protocol should be repeated trying to avoid air bubbles or making a thinner PDMS layer the next time.
Step 12	All objects stick to the surface.	Passivation did not work properly or the passivation agent (Pluronic F-127) does not work per se.	The protocol should be repeated or another protocol for passivation has to be established.
Step 12	Objects are defocusing.	The PDMS layer is too thick or air bubbles are in the sample	The protocol should be repeated. An alternative way to adjust the height of the measurement sample is the usage of spacer particles.
Step 12	There is a flow in the sample.	The PDM layer does not seal the sample properly or there are air bubbles in the sample.	If the flow is not gone after a waiting time of ca. 15 min, the protocol should be repeated.

Figure 7

Table 1 Troubleshooting