Magnetic stage with environmental control for optical microscopy and high-speed nano- and microrheology.

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Abstract

A novel design of a low-field magnetic stage for optical microscopy of droplets and films within a controlled environment is described. The stage consists of five magnetic coils with a 3D magnetic sensor in a feedback control loop, which allows one to manipulate magnetic nano and micropores with microTesla fields. A locally uniform time-dependent field within the focal plane of the microscope objective enables one to rotate the probes in a precisely set manner and observe their motion. The probe tracking protocol was developed to follow the probe rotation in real time and relate it with the viscosity of the host liquid. Using this magnetic stage, a method for measuring mPa-s-level viscosity of nanoliter droplets and micron thick films in a 10-20 second timeframe is presented and validated. The viscosity of a rapidly changing liquid can be tracked by using only a few visible probes rotating simultaneously. Vapor pressure and temperature around the sample can be controlled to directly measure viscosity as a function of equilibrium vapor pressure; this addresses a significant challenge in characterization of volatile nanodroplets and thin films. Thin films of surfactant solutions undergoing phase transitions upon solvent evaporation were studied and their rheological properties were related to morphological changes in the material.

1 Introduction

Rheological analysis of pico/nano liter samples presents a significant challenge [1-7]. Many polymers and biofluids rapidly react to the environmental conditions by changing their rheological properties[8]. Furthermore, in biofluids such as cellular, mucosal and tissue fluids, the fluid viscosity varies over time, making it challenging to quantify and analyze these changes. Rheology of biopolymer solutions and gels depends on the concentration, level of cross-linking, oxygen content, temperature, pH, and many other environmental parameters. Drastic thickening of fluids in a short time interval often results in the far-reaching consequences. For example, sickle cell disease results in the increase of the cytosolic viscosity inside the red blood cells [9, 10]; polymerization of fibrinogen leads to fibrin clot formation and wound healing [11-13]. Polysaccharides that gelate in fractions of a second serve as reactive extracts and cuticular building blocks in animals and insects [14-16] and have practical applications for manufacturing fibers and films [17-21]. These broad applications and abundance of systems that rapidly change their rheological
behavior, call for the development of new methods and instrumentation for their analyses and characterization.

Current rheological methods that are used to study microscopic droplets are based on suspending probes in the studied medium and tracking their behavior. These methods fall under two categories: passive – when the small probes are subject to thermal excitation and drift in the fluid on their own – and active – when the probes are actuated by external forces [22-24]. Advanced passive microrheological techniques have been used in studies of complex fluids (see [25] for review) and various biological media (see [26] for review) by applying models of particle motion in viscous or viscoelastic media. The method, however, assumes a purely diffusive motion of the probes and the analysis of experimental data is significantly complicated by the presence of flow, heterogeneity of the sample, or proximity to the substrate [27]. Active microrheological techniques have an advantage of controlling the load on the probes, allowing for the stress-controlled studies. The methods of force application range from atomic force microscopy tips for surface probing [28, 29], to optical tweezers on highly refractive probes in highly transparent materials [30-33], to magnetic actuation or rotation of magnetic probes [4, 34-43]. The latter two methods are able to apply the load remotely and without any mechanical contact with the probe. Optical tweezers, however, rely on high-intensity light to apply the force, which can significantly increase the local temperature near the probe and alter the sampled medium. Magnetic actuation, meanwhile, is able to apply a load directly on the magnetic probes without any disturbance of the medium, making this technique non-destructive and thus highly attractive.

Probing of materials properties with magnetic nano and micropores was pioneered almost half a century ago [44, 45] and with the progress in nanotechnology and microfluidics, it shows a growing interest in the community of researchers dealing with the analysis of small samples [3, 6, 7, 36-39, 46-53]. Recent studies show that accurate tracking of a single probe can yield accurate rheological measurements [4, 34-40]. While such measurements are successfully used to study highly viscous liquids, they are difficult to perform with low viscous liquids, such as dilute aqueous solutions of biopolymers, surfactants, and salts. For such liquids, the torque applied on the probes must be low – e.g., for a nickel nanorod of 200 nm in diameter and 10 μm in length in water of 1 mPa·s viscosity, the torque needs to be roughly $10^{-17}$ N·m. To apply such a torque on such a rod, the applied magnetic field must be on the order of 100 μT. When magnetic fields are microTesla weak, control over the movement of magnetic probe is hindered by the presence of Earth’s magnetic field, which ranges from 50 μT to 100 μT. The applied magnetic field is disturbed by this bias thus significantly affecting the readings of magnetic sensors that pick up responses of magnetic probes. Therefore, for accurate measurements, the magnetic field needs to be accurately controlled and Earth's magnetic field needs to be actively cancelled.

In this paper, we address all these challenges by offering a new design of a magnetic microTesla stage for optical microscopy of droplets and films within a controlled environment. The earlier publication of our group dealt with a rotation stage providing some milliTesla magnetic fields[40]. Here we discuss a new design, integrating magnetic coils with a 3D magnetic sensor with the feedback control which allows us to significantly decrease the field. At the same time, the stage offers the user a flexibility to control the 3D field configuration and is able to create a locally uniform field within the focal plane of the microscope objective. The optical cell equipped with the environment control circuit is fit under the Olympus BX51 upright microscope. We illustrate robustness of this stage by studying viscous properties of liquids with a high-speed rheological technique based on Magnetic Rotational Spectroscopy [4, 36, 54, 55], using magnetic nano- and microrods as the probes.
2 DESIGN OF THE MAGNETIC STAGE

2.1 DESIGN OVERVIEW

Designing a stage for manipulation of magnetic probes with a microTesla rotating magnetic field in the focal plane of the microscope stage, the following challenges need to be addressed.

1) The magnetic field of Earth is three-dimensional (3D). It has to be cancelled in the focal plane of the microscope with an equal and opposite field. The cancelling 3D field has to be controlled on demand to guarantee that at different geographic locations having different Earth’s magnetic field the stage will work correctly.

2) The magnetic field in the region of interest must be uniform and the magnetic gradient, which may cause a translational motion of magnetic probe, must be minimized.

3) The composition and humidity of the gas around the sample must be maintained to be the same during measurements or changed on demand in a controlled fashion using an environmental chamber.

The design that addresses all three challenges is schematically presented in Figure 1. Five independently driven magnetic coils surround the magnetic sensor, which measures the three orthogonal components of magnetic field. It thus becomes convenient to work in a Cartesian coordinate system with the origin located at the sensor center and axes aligned with the axes of the sensor. The xy plane thus becomes parallel to the ground. Coils 1 and 3 are placed in tandem and equidistant from the sensor, such that their axes are parallel to the x-axis. Similarly, the axes of coils 2 and 4 are parallel to the y-axis. Due to the spatial constraints associated with the need to accommodate an objective of a microscope and an environmental chamber, coil 5 is positioned directly underneath the sensor with its axis parallel to z-axis. The produced 3D magnetic field satisfies all three criteria.

Figure 1. (A) A schematic of 5 independent magnetic coils each producing its own magnetic field ($B_1$-$B_5$). The sensor (green square) measures and records the resultant three vector components of the magnetic field ($B_x$, $B_y$, $B_z$). Coils 1 and 3 are aligned relative to the x-axis, coils 2 and 4 are aligned relative to the y-axes, and coil 5 is aligned relative to the z-axis (B) Basic elements of the magnetic stage with the environmental control.
The design of the stage was implemented in an instrument presented in Figure 2. Five coils with a magnetic sensor create the required magnetic field at the location of the sample (Figure 2A, B). The coils are driven by a signal generator software written in LabView for a NI-DAQ voltage generator coupled with a power amplifier. The produced magnetic field is measured by the magnetic sensor and fed to the software in a feedback loop; the software calculates what signal to send to each magnet. This way, any constant magnetic field can be created to counter any ambient field. This constant component is superimposed with a rotating magnetic field of a desired amplitude and frequency to rotate magnetic probes. By observing the motion of the probes with a camera coupled with a microscope, the magnetic field can be adjusted in real time to induce the desired mode of rotation. For further analysis of the probe rotations, LabView VISION package algorithms are used.

The humidity and temperature of the sample on the magnetic stage are stabilized with an environment control system that pumps a gas of desired composition and humidity over the surface of the sample at a low flow rate. During the experiment, a droplet of the sample is placed in the chamber allowing evaporation to occur. The vapor mixes with supplied humidified nitrogen and exits the chamber. The sample thus evaporates until its equilibrium vapor pressure becomes equal to the vapor pressure of the supplied nitrogen-vapor mixture.

The engineering implementation of the system is described below.
Figure 2. Schematics (A, C) and a photograph (B) of the magnetic stage with environmental control. (A) Basic elements of the magnetic stage with the environmental control. (B) A photograph of the optical cell with environmental control surrounded by five magnetic coils mounted under a microscope. (C) A cross-section schematic of the supply system of nitrogen of controlled humidity to the optical cell (not to scale). The inner chamber consists of an open-ended cylinder; the wall of this cylinder is schematically; the height of the inner chamber is slightly smaller than that of the outer chamber, leaving a small gap for the gas to flow through. As the gas moves over the inner cylinder, it gets mixed with the water vapor from the sample.
2.2 **Details of the Engineering Implementation**

The above design is implemented in the following way (Figure 2A). A microscope-compatible acrylic base plate holds the four driving coils (HobbyEngineering) firmly attached, each facing the center of the stage. Resting in the center of the stage is a 3D printed mount that holds the fifth out-of-plane coil directly underneath the magnetic sensor (HMC5883L, Adafruit), which measures the three components of the magnetic field. The sensor is covered by a thin cover glass, serving as a floor of the measuring chamber. A slide with magnetic probes suspended in the studied sample is placed directly on the chamber floor, thus ensuring the distance between the sample and the sensor is under 1 mm. Thus, the field measured by the sensor has the same magnitude and direction as the field that propagates through the sample and acts on the probes. The chamber is covered by a transparent cap with the inlet and outlet ports for the circulating gas. This cap seals the environmental chamber and allows one to observe the motion of magnetic probes with a microscope.

The dimensions of the system were designed with the following spatial constraints. To produce the smallest gradient with a tandem two-coil configuration, the distance between the coils must be minimized. At the same time, however, the magnetic sensor (19x20 mm) and a microscope objective (d=32 mm) must be able to fit between the coils. For that reason, the driving coils (d=25 mm) were mounted 33 mm apart. The mount, which has to hold the out-of-plane coil and the sensor, had the diameter of 29 mm. The diameter and the height of the environmental chamber were limited by the diameter of the mount and the working distance of the objective, respectively. To fit the mount, the diameter of the chamber was chosen to be 25 mm. The working distance of a 50x Olympus lens is 10 mm, thus the height of the chamber was made to be 9 mm; the height of the inner chamber cylinder was made 8.5 mm.

To verify whether the coil separation distance yields an acceptable field with minimal gradient, finite element analysis was performed with COMSOL software. Two coils of radius \( R \), identical in geometry to the coils used in the setup, were placed uniaxially with a separation distance \( d \). The magnetic field and its gradient generated by the coils were calculated for different \( d/R \) ratios. For the ratio used in the instrument (\( d/R = 2.6 \)), the both variables were plotted on a 2D slice through the central axis (Figure 3A). For other values of \( d/R \), the magnetic field and the magnetic gradient along the center axis were plotted in Figure 3B and C, respectively. From this analysis, it is clear that the coil configuration used in the instrument yields zero gradient at the center between the two coils and a uniform magnetic field for several millimeters around the center point.

A similar analysis was performed to verify that a single z-coil produces an acceptable region of uniform field at the sample (Figure 3D). The analysis demonstrates that at a distance of 0.08·R away from the coil, which is the distance between the coil and the sample in the instrument, the field is uniform over roughly 0.2·R. In the instrument, this corresponds to a region of 5 mm in diameter, which is larger than the size of a typical sample droplet. This analysis demonstrates that the magnetic field produced by the magnetic stage is uniform and gradient-free in all three directions at the sample location.
Figure 3. Results of a finite element analysis of magnetic field generated by: (A-C) a pair of coils and (D) a single coil. (A) An arrow plot of the magnetic field superimposed with a heatmap plot of the magnetic gradient generated by a pair of coils of radius R separated by a distance \(d = 2.6 \cdot R\). The geometry is similar to the coil configuration of the instrument. The arrows in the arrow plot are scaled logarithmically with a range quotient of 100. (B) Plots of the magnetic field in the axial direction along the axis of the coils for several separation distances. The position is normalized by \(d/2\) and the zero coordinate is the midpoint between the coils. (C) Plots of the magnetic gradient in the axial direction along the axis of the coils for several separation distances. The position is normalized by \(d/2\) and the zero coordinate is the midpoint between the coils. (D) Plots of a magnetic field created by a single coil in the axial direction along a radial coordinate. The radial coordinate is normalized by the coil radius. 

To control the environment around the studied sample, an environmental control module was implemented. The environmental control of the sample is performed by flowing a gas of controlled composition and humidity through the environmental chamber (Figure 2C). After passing through the chamber, the gas is released directly into the atmosphere through an aperture \(d = 2\text{mm}\) that simultaneously serves as an access point for the user to the sample. The smallest allowable flow rate of the gas through the environmental chamber is thus controlled by the diffusive flux of air from the atmosphere back into the chamber. The evaluation of Peclet number and an experimental verification (details in Supplementary Information 1) place the low-end constraint of the flow rate at 1 Standard Cubic Foot per Hour \(\text{1 SCFH} = 7.8 \cdot 10^{-6} \text{ m}^3/\text{s}\). The upper constraint of the flow rate is due to the induced flow in the sample at the liquid-gas interface. To lower the effect of the induced flow, we separated the environmental chamber into two – inner and outer in Figure 2C - with a cylindrical wall. The wall lowers the flow of gas directly over the surface of the sample, yet still allows the sample vapors to mix with the supplied gas. With this wall, the maximum flow rate was experimentally determined to be 2 SCFH or \(15 \cdot \).
The workable range of the flow rates of supplied gas was thus determined to be between 1 and 2 SCFH (7.8 \times 10^{-6} m^3/s and 15 \times 10^{-6} m^3/s, respectively).

In the current study, the environmental chamber was supplied with a nitrogen gas – water vapor mixture. For this purpose, the design of Ref.[56] was adapted and fitted with a humidity sensor (HIH-3040, Sparkfun) at the input to the environmental chamber. The details of the supply system are discussed in Supplementary Information 1.

By introducing a heating element into the environmental chamber, we are also able to set the temperature of the sample. A polyimide film-based flexible heating element (KA-808, Omegalux) was placed underneath the sample. A DC regulated power supply (CSI12001X, Circuit Specialists) was connected to the heating element and controlled with a LabView program to maintain the temperature on the glass slide. The temperature on the slide was measured with a thermocouple connected to a temperature controller (TC-3300, CAL Controls LTD) and used for feedback control of the temperature. The details on this system can be found in Refs. [40, 57]. During heating, the magnetic field generated by the current in the heating element was negligible and was not detected by the magnetic sensor.

2.3 COMMUNICATION WITH A COMPUTER

A LabVIEW program produces the signal for the magnetic coils via an analog PCI board (NI-PCI 6722). The signal then gets amplified by five magnetic drivers. Our setup uses custom-made magnetic drivers that are based on the power operational amplifiers (PA02, Apex); alternatively, commercially available magnetic drivers can be used.

The magnetic sensor communicates with the LabView program via the I2C protocol and is able to record up to 75 measurements per second. The humidity sensor communicates with the programs via analogue voltage and is able to produce a measurement every two seconds.

2.4 MAGNETIC SIGNAL GENERATOR SOFTWARE

In order to create a desired magnetic field, the magnetic controller program calculates the voltage to apply over each magnetic coil. There are five coils that create a magnetic field; the 3D magnetic sensor measures its three orthogonal components in the X, Y, and Z directions at the location right under the sample. (Figure 1A).

The naïve approach for setting the magnetic field is to assume identical coils, perfect alignment along their respective axes, and ignore the ambient field. We demonstrate in Figure 5A) that this approach yields unacceptably inaccurate results at low fields.

The coils cannot be perfectly aligned relative to their axes and are not perfectly identical. Therefore, one cannot a priori assume the field uniformity; each coil contributes differently to all three components of the field. Due to the Biot–Savart law, these contributions are linear with respect to the voltage over the coil. Assuming that any ambient magnetic field is constant and does not depend on the voltages over the coils, each component of the magnetic field is expressed as the sum of the ambient field and a linearly scaled voltage over the coil:
\[ B_x = B_{x0} + a_1 V_1 + a_2 V_2 + a_3 V_3 + a_4 V_4 + a_5 V_5 \]
\[ B_y = B_{y0} + b_1 V_1 + b_2 V_2 + b_3 V_3 + b_4 V_4 + b_5 V_5 \]
\[ B_z = B_{z0} + c_1 V_1 + c_2 V_2 + c_3 V_3 + c_4 V_4 + c_5 V_5 \]  

(1)

where \( B_x, B_y, \) and \( B_z \) are the components of the total magnetic field measured by the sensor, \( B_{x0}, B_{y0}, \) and \( B_{z0} \) are the components of the constant ambient magnetic field, \( V_n, n = 1,2,3,4,5 \) are the voltages supplied to the magnetic drivers, and \( a_n, b_n, \) and \( c_n \) are the calibration coefficients for the five coils.

In order to find the coefficients \( a_n, b_n, \) and \( c_n, \) the program communicates with the 3D magnetic sensor and performs the following series of steps:

1. Sends a zero voltage over each coil and collects a magnetic field measurement. We thus obtain the value of ambient magnetic field (\( B_{x0}, B_{y0}, \) and \( B_{z0} \)).

2. Incrementally increases voltage over each coil, while keeping all other voltages zeroed (e.g., \( V_1 = 0.1V, 0.2V, 0.3V \ldots 1V, \) while \( V_2 = V_3 = V_4 = V_5 = 0V \)). At each step, the program collects a magnetic field measurement. It thus obtains a linear plot of each component of the magnetic field versus voltage applied over each coil.

3. Linearly fits each plot, thus finding each of the coefficients (\( a_n, b_n, \) and \( c_n \)) in equation (1) (Figure 4).

![Graph](image)

**Figure 4.** (Top) A typical example of calibration plots of each component of the magnetic field versus voltage applied over each coil obtained as a result of the calibration algorithm. (Bottom) The table of the results of linear regressions of each plot. \( B_0 \) is the average y-intercept and is interpreted as the ambient magnetic field in a given direction. \( m_n \) is the slope of the field produced vs. voltage supplied to coil \( n \) in a given direction. \( m_n \) in \( x, y, \) and \( z \)-directions correspond to \( a_n, b_n, \) and \( c_n \) coefficients in equation 1.
Once the coefficients are obtained, equations (1) must be solved for \( V_1, V_2, V_3, V_4, \) and \( V_5 \) at given values of \( B_x, B_y, \) and \( B_z. \) This provides a challenge, however, since there are three equations and five unknowns. The number of unknowns is reduced to three by taking into account the fact that for the magnetic gradient to be minimal, the voltage over the coils must be applied in such a way, that both coils contribute equal components of the magnetic field in the direction of their alignment axis (i.e. the axis specified by the magnetic sensor). Mathematically, this statement is expressed as

\[
a_1V_1 = a_3V_3 \quad b_2V_2 = b_4V_4 \quad \Rightarrow \quad V_3 = \frac{a_1V_1}{a_3} \quad V_4 = \frac{b_2V_2}{b_4}
\]

Substituting equation (2) into equation (1), and solving the resulting system of equations, the supplied voltages in terms of components of the desired magnetic field are obtained as

\[
\begin{bmatrix}
V_1 \\
V_2 \\
V_5
\end{bmatrix} = \begin{bmatrix}
a_1 & a_2 + a_4 \frac{b_2}{b_4} & a_5 \\
a_3 & b_1 + b_3 & 2b_4 \\
a_3 & a_1 \frac{c_1 + c_3}{b_1} & b_2 \frac{c_2 + c_4}{b_4}
\end{bmatrix}^{-1} \begin{bmatrix}
B_x - B_{0x} \\
B_y - B_{0y} \\
B_z - B_{0z}
\end{bmatrix}
\]

The user is then free to specify any magnetic field. The software then calculates the voltages to send to each coil to create this field. The profile of the time-dependent components of the desired magnetic field is created using a built-in LabVIEW algorithm called “Basic Function Generator.” The user has a choice of a sine, square, sawtooth, or a triangle wave with adjustable parameters, such as the amplitude, frequency, and bias. This feature allows the user to quickly and accurately define the desired spatial and time profile of the magnetic field. Furthermore, the program can merge a series of waveforms with different parameters together – e.g. a sine wave with an increasing frequency.

In many applications, a 2D rotating field is required to manipulate magnetic particles \([4, 55, 58-60].\) We thus demonstrate the applicability of the calibration algorithm by cancelling out the ambient magnetic field of 38μT (Earth’s magnetic field in Clemson, SC) and imposing a rotating field of 100μT in the XY plane (Figure 5B). It is apparent that with the applied calibration, the system is able to set a precisely defined magnetic field, accounting for Earth’s magnetic field and the slight misalignment of magnetic coils.
3 EXPERIMENTAL DEMONSTRATION: PROBING FLUID VISCOSITY WITH MAGNETIC RODS

We demonstrate the robustness and variability of the instrument by performing microrheological measurements of the viscosity standards under different conditions. To demonstrate the effectiveness of the temperature control, we vary the temperature of the sample, while controlling sample evaporation. To demonstrate the effectiveness of humidity control, we vary the environmental humidity, while maintaining a constant temperature.

To determine the viscosity of the sample, we modify the method of magnetic rotational spectroscopy (MRS) with ferromagnetic rod-like probes. The method allows to accurately detect viscosity changes of the liquid medium by tracking the changes of frequency of rotation of magnetic probes. The experiment consists of measuring the probe rotation frequency at the known viscosity and environmental conditions, then changing the environmental conditions, and tracking the changes in rotation behavior of the probe. We thus obtain the plots of viscosity as a function of temperature and humidity (Section 3.4).

3.1 MEASURING VISCOSITY

A magnetic field \( \mathbf{B} \) acting on a ferromagnetic rod with a magnetic moment, \( \mathbf{m} \), exerts a mechanical torque \( \mathbf{\tau}_m = \mathbf{m} \times \mathbf{B} \) [61, 62]. If the rod is suspended in a viscous medium of viscosity \( \eta \), the medium responds to the rod rotation with a torque \( \mathbf{\tau}_v = \eta \dot{\gamma} \hat{e} \) opposing its rotation, where \( \dot{\gamma} \) is the angular velocity of the rod, \( \gamma \) is the angle that the magnetic moment \( \mathbf{m} \) makes with a fixed axis \( x \), \( \hat{e} \) is the unit vector pointing perpendicularly to the plane of rotation, and \( \hat{e} \) is the drag coefficient, which is defined for a rod of length \( l \) and diameter \( d \), \( l/d >> 1 \), as[63, 64]: \( \gamma = 2\pi l^3 \left( 6\ln\left(2 \frac{l}{d}\right) - 3 \right)^{-1} \). After writing the balance of magnetic and viscous torques as \( \mathbf{m} \times \mathbf{B} = \eta \dot{\gamma} \hat{e} \) and solving this equation, one obtains

Figure 5. Demonstration of effectiveness of elimination of the ambient magnetic field. (A) A measured profile of a generated magnetic field with \( a_1 = a_3 = b_2 = b_4, \text{all others} = 0 \). The offsets are caused by Earth's magnetic field and unwanted oscillations in the z-component are caused by an imperfect alignment of magnets. (B) A measured profile of the generated 100μT rotating magnetic field in the xy-plane after calibration. The offsets and unwanted oscillations are removed.
trajectories of the rod endpoints during rotation. These trajectories depend on the viscosity of the medium. Knowing the rod parameters \( m \) and \( \gamma \) and knowing the angular frequency of the applied field \( \omega \) and \( B \), one can identify the viscosity of the liquid[4]. This model and experiments reveal a surprisingly complex behavior of magnetic rod in the 2D rotating magnetic field[4]. For the in-plane rotation, the torque balance is reduced to the following equation[34, 35, 37, 65]:

\[
\dot{\phi} = -\omega_c \sin(\varphi - \omega t)
\]  

where \( \omega_c = mB / (\eta \gamma) \) is the critical frequency of the rod rotation[4]. When the frequency of the rotating magnetic field is below this critical frequency, the probe is synchronously rotating with the field at frequency \( \omega \), and magnetic moment makes a constant angle with the field, \( \theta = \varphi - \omega t \). The angle \( \theta \) is obtained by solving transcendental equation \( \omega = \omega_c \sin \theta \). When the frequency of the rotating magnetic field is set above the critical frequency, \( \omega / \omega_c > 1 \) the transcendental equation does not make any sense, i.e. the solution \( \theta = \text{const} \) does not satisfy equation (4), suggesting that the probe cannot rotate synchronously with the field. Surprisingly, the rod keeps rotating in the same plane, but the trajectory of the rod end acquires an oscillatory time periodic component. In this asynchronous rotation, the rod keeps revolving periodically with the period of the rod oscillation \( \omega_r \) depending on the frequency of applied field, \( \omega_r = \omega_r(\omega) \). Recently, equation (4) was generalized on viscoelastic fluids and gels assuming the same form of the drag coefficient [36, 38, 66, 67].

Traditionally, the Magnetic Rotational Spectroscopy relies on the analysis of spectrum \( \omega_r = \omega_r(\omega) \) [4, 67] when one needs to change the frequency of rotating field in a broad range of frequencies from below to above the critical frequency covering all the features of rod rotation [37, 65-68]. The major shortcoming of this methodology is the inability to probe rheology of materials with the properties rapidly changing with time. If the critical frequency of the system is 1Hz, which is typical for many liquids-probe systems [4, 67], and each data point is collected during at least 10 revolutions of the rod, a single measurement would take at least 10 seconds. To study the spectrum \( \omega_r = \omega_r(\omega) \), one needs at least 10 measurements at 10 different frequencies; this requires, \( 10 \times 10 = 100 \) seconds. To collect statistics, i.e. repeating the experiment 10 more times, one needs \( \sim 17 \) minutes. This is a lower-end estimate, which assumes that no time is required to switch between frequencies; a realistic experiment will take longer time than that. Thus, while the methodology is viable for probing liquids with non-changing properties, it does not work for liquids that change their properties at this time scale. There are many examples where such viscosity characterization is challenging, including evaporating solutions and samples undergoing chemical reactions[55][69, 70].

Fortunately, one can significantly speed up the measurements by developing a particle tracking algorithm and fitting the experimental trajectories of magnetic rods with the theoretical ones predicted by equation (4). There is a single fitting parameter in this model, \( \omega_c = mB / (\eta \gamma) \), which allows one to make this analysis straightforward. For a system with a critical frequency of 1Hz, a single measurement would take only 10-20 seconds. Using few visible rods rotating simultaneously in the focal plane of observation, one can gather a statistics in one shot. This allows for tracking the viscosity of a rapidly changing liquid – something impossible to do with MRS. We have used this idea and a first generation of
the 5-coil setup to study the magnetic properties of ferromagnetic probes.[34] This idea was further developed and is now illustrated here using the magnetic stage with environmental control. We demonstrate the effectiveness of a single frequency measurement in both stable liquids and in liquids with rapidly changing properties. The probes of different length can be effectively used with this stage; we studied probes with the length ranging from several micrometers to millimeters. Nanorods were prepared from nickel using electro-chemical template synthesis in accordance with the protocol outlined in Ref. [34]. As an example of nanoprobes, the 340nm diameter and 15-20μm length nanorods are used in this paper. As an example of microprobes, the 50μm diameter and 1-2mm length rods are utilized; a stainless steel SUS304 wire (Tokusen) was cut in pieces to make these microprobes.

3.2 VIDEO ANALYSIS

An optical microscope equipped with a camera allows one to track the 100 nm thick and a few microns long nanorods[4]. The imaging is often complicated by non-uniform backgrounds and foreign objects, which are abundant in microrheologically complex samples such as biofluids. Moreover, in samples with an open liquid-air interface, one often encounters fluid flows drifting the probes through the sample. Thus, one has to follow not only the probe rotation, but also its translation movement. All these challenges are resolved with a custom code based on LabVIEW Vision Development Module. This is a convenient solution, since the user can adjust video analysis parameters on the fly to obtain best results.

The nanorod image extraction algorithm is as follows. First, the user specifies the region of interest where the software should find the nanorod; all objects outside of this region are disregarded. A binarized image is then created using either constant threshold values or background correction via IMAQ Local Threshold algorithm. Binarization of the image sometimes causes artificial holes to appear in objects; these holed are automatically filled in using IMAQ FillHole algorithm to insure correct further analysis. Any analysis of objects that are not fully imaged (i.e. located at the edge of the frame) would result in faulty results; such objects are deleted using IMAQ RejectBorder algorithm. Foreign objects, such as cells or dust, or noise in the image may result in faulty analysis; these imperfections are typically either smaller or larger than the nanorod and are thus filtered out by size using IMAQ RemoveParticle algorithms. As a result of these steps, a new image is created where only the nanorods of interest remain. For these nanorods, the orientation angle, the position, and the length are extracted using IMAQ Particle Analysis Report and IMAQ Clamp Max algorithms, respectively. To prepare for the analysis of the next frame, the region of interest is programmatically shifted to the coordinates of the nanorod and rotated to align with the orientation of the nanorod.

This process is applied to every frame in the specified segment of the video. In such, the algorithm tracks the nanorod in each frame of the video and rejects any undesirable objects. The measurements of apparent length of the nanorod image in each frame provides a metric for analysis of the angle of declination of the nanorod axis from its original plane of rotation. The effectiveness of the algorithm is illustrated in Figure 6. In this example, the angle of rotation $\varphi$ increases and periodically oscillates with time. The apparent length of the nanorod remains constant suggesting that the nanorod rotates in the focal plane. The program struggled with one frame (number 134) and produced a shorter length than expected. The algorithm, however, was able to successfully analyze all the consequent frames providing all necessary data for further analysis.
Once the data is extracted, the angular trajectory of the rod $\varphi(t)$ is fitted with equation (4) using the Levenberg–Marquardt function in LabView with $\omega_c$ as an adjustable parameter. The volume and the $\gamma$ parameter of the probe are calculated using the average nanorod length $l$ extracted from the video and diameter $d$. The magnetic moment $m$ of the probe is then calculated from the calculated volume and the saturation magnetization $M_s$ of the material as $= 4\pi d^2 l M_s$. Using these values, the viscosity is calculated as $\eta = mB / (\omega_c \gamma)$. For this particular example in Figure 6, the viscosity was measured to be 2.8 mPa·s.
Figure 6. (A) An example image of the nanorod superimposed with the measured results for that particular frame. (B) A binary image of the frame with imperfections due to noise, uneven background, and present particles; the imperfections are removed by the developed software. (C) A gallery of frames showing a drifting nanorod with several foreign objects; (D) The extracted angle $\phi$, (E) apparent length $l$, and (F) the in-plane coordinates $(x,y)$ (in pixels) of the rod center mass for each frame.
3.3 CONTROL OF THE OUT-OF-PLANE ROTATION

During experimental observations of asynchronous rotation of ferromagnetic probes, we have detected that sometimes the apparent length of the observed nanorod changes. This implies that the nanorod declines out of the plane of rotation of the magnetic field (Figure 7). This behavior was most often observed when the coil calibration became inaccurate, as a result of either a change in the ambient magnetic field – due to accidental rotation of the set-up or movement of a magnetic object in the lab – or heating up of the coils due to an overly high current. This, in turn, led to an inaccurate cancelation of the ambient magnetic field and resulted in a slight bias of the rotating field. The problem was easily resolved by recalibrating the system and did not significantly hamper the rheological measurements. It did, however, allow us to document an interesting regime of nanorod rotation that was theoretically predicted for soft magnetic nanorods [71, but never experimentally observed.

The explanation of this phenomenon is as follows. The net magnetic torque on the nanorod is always directed along the shortest path between the nanorod and the magnetic field. When the vectors of magnetic moment and external rotating field form an angle $\theta$ that is much smaller than 180 degrees, the net torque $\mathbf{m} \times \mathbf{B} \propto \sin \theta$ drives the nanorod to rotate in the focal plane as shown in Figure 7A and B. When the magnetic moment is antiparallel to the applied field, the magnetic torque is $\mathbf{m} \times \mathbf{B} \propto \sin \theta \approx 0$, and any out-of-plane bias field changes the torque direction causing the nanorod to decline from the focal plane as shown in Figures 7C, D, and E. When a nanorod rotates near its critical frequency, it spends a relatively long time in the direction antiparallel to the magnetic field. At this position, the nanorod is very sensitive to any small out-of-plane perturbations of magnetic field.

![Figure 7](image)

*Figure 7. (Bottom) A gallery of snapshots of a ferromagnetic nanorod rotating at the onset of the out-of-plane rotation regime. (Top) A series of schematics to explain the behavior of the rod by illustrating the 3D orientations of the magnetic field $\mathbf{B}$ (orange), the orientation of the magnetic moment of the nanorod $\mathbf{m}$ (blue), the angle $\theta$ along the shortest path between $\mathbf{m}$ and $\mathbf{B}$, the projection of $\mathbf{m}$ on a plane parallel to the rotation of the field (grey), and the angle $\phi$ that the projection makes with a stationary axis. The magnetic field rotates in the focal plane, while the nanorod and associated magnetic moment vector comes out of plane. The more the nanorod comes out of plane, the shorter its projection on the focal plane becomes. (A, B) the angle $\theta$ is relatively small and lie in the focal plane, so the nanorod rotates in the same plane. (C) As the angle $\theta$ approaches 180° a small out-of-plane bias magnetic field makes the angle $\theta$ to come out of the plane, causing the nanorod to stick out of the plane.*
An extracted trajectory $\varphi(t)$ and associated apparent length $l(t)$ of the nanorod are shown in Figure 8. In contrast to Figure 6, where the apparent length does not change during the observation time, in Figure 8, the apparent length of the nanorod changes with time. Quantitatively, one can characterize the level of nanorod deviation from the plane of field rotation by introducing the declination angle $\beta = \arccos(l_{apparent}/l)$, where $l_{apparent}$ is the length of visible projection of the nanorod on the focal plane, i.e. the plane of field rotation and $l$ is the actual length of the nanorod. The shorter the apparent length the greater the inclination angle of the nanorod axis with respect to the plane of field rotation; the angle $\beta$ approaches $\pi/2$ as the apparent length $l_{apparent}$ goes to zero and approaches zero as the apparent length approaches the actual length, $l_{apparent} \approx l$ implying that the rod is closer to the plane of rotating field. The graphs in Figure 8 confirm that the nanorod is subject to the out-of-plane precession. The plots reveal that until frame 400, the nanorod asynchronously rotates in plane of the field rotation. At frame 400, out-of-plane oscillations (oscillation in angle $\beta$) appear and increase until frame 650, when the nanorod apparently switches to the out-of-plane synchronous rotation: the trajectory $\varphi(t)$ looks linear as it should be for a synchronous rotation of the nanorod with a rotating field. After frame 650, however, the apparent length of the nanorod oscillates around a declination angle of 50 degrees. Thus after frame 650, the nanorod rotation has a precession component as well as an oscillatory component around the precession cone.
A qualitatively similar out-of-plane rotation of a nanorod driven by a planar rotating magnetic field was predicted for a magnetically soft nanorod [71]. As shown in Ref. [71], the nanorod magnetic moment does not have to align with the nanorod long axis and is able to decline under the influence of an external 2D magnetic field, i.e. a planar rotation of the nanorod is inherently unstable and no bias field is needed to push the nanorod out of plane. The tendency of the magnetic moment to align with the long axis of the rod is quantified by the materials parameter, the energy of magnetic anisotropy, $K$ [72]. When the material is magnetically soft, $K$ is small, the direction of the magnetic moment relative to the long axis of the rod can be easily changed; when the material is magnetically hard, $K$ is large, the direction of the magnetic moment can only be changed with a strong applied magnetic field. The nanorods used in this study were shown to be magnetically hard with the energy of magnetic anisotropy $K=22\times10^3\,\text{J/m}^3$ [34].

Cimurs and Cebers showed that the out of plane rotation of a magnetically soft nanorod describes a cone of the angle $\gamma$ with the normal vector to the focal plane [71]. This angle is related to the $K$ parameter as [71]:

\[
\sin(\gamma) = K / K_{\text{sat}}
\]
1−\cos^2 \gamma = \left(\frac{\omega_c}{\omega_H}\right)^2 \left(1−\left(\frac{B}{B_a}\right)^2\right) \quad (5)

where \omega_H is the frequency of rotating magnetic field, \(B\) is the magnitude of the applied magnetic field, \(B_a=K/M_r\), and \(M_r\) is the remanent magnetization of the nanorod material. Using the experimental values of the presented case: \(\omega_c = 1.14 \text{ Hz}, \omega_H = 2 \text{ Hz}, B = 100 \mu\text{T}, M_r = 140*10^3 \text{ A/m}, \text{ and } K=22*10^3 \text{ J/m}^3\) – we solve equation (5) to obtain the cone angle \(\gamma = 34^\circ\). This theoretical angle differs from the experimentally observed cone angle, \(= 90^\circ - \beta = 40^\circ\). Moreover, if we attempt to solve equation (5) for \((B/B_a)^2\), using \(\gamma = 40^\circ\), and the same experimental parameters, we obtain \((B/B_a)^2 < 0\), which is physically impossible.

This discrepancy between theory and experiment comes from the assumption that the field is rotating in plane without any bias and the nanorod is magnetically soft. Thus, the Cimurs - Cebers theory is not sufficient to explain these experiments: there is a slight bias in the field and the nanorod material is magnetically hard. To understand the contributions of both magnetic anisotropy and an out-of-plane magnetic field, a thorough study needs to be conducted to analyze the nanorod behavior as a function of these parameters.

### 3.4 Micro and Nanorheology

The developed magnetic stage is able to work with different magnetic particles ranging from hundreds of nanometers to millimeters. Stainless steel microrods (50 \(\mu\text{m}\) in diameter) were used to replicate the tabulated viscosity of aqueous ethylene glycol solutions of varying concentrations providing viscosities ranging from 1 mPa\(\cdot\)s to 10 mPa\(\cdot\)s. The probes were placed in deionized water and the critical frequency \(\omega_{cr}\) was measured. Since the viscosity of water is known, \(\eta = 1 \text{ mPa}\cdot\text{s}\), and applied magnetic field is known as well, this measurement has served as a reference specifying the \(m/\gamma\) ratio. Then, the same probes were used to measure the critical frequency of solutions with different concentrations of ethylene glycol. Each solution gives its own critical frequency \(\omega_{cr}^{solution}\) from which the solution viscosity was calculated. The results of these experiments are summarized in Figure 8A, where the tabulated viscosity is shown as a solid line. The experimental points fall on this line nicely confirming the validity of the developed protocol.
Figure 9. A) Profile of the measured viscosity of prepared aqueous ethylene glycol (EG) solutions using stainless steel SUS304 microrods. Tabulated values are taken from [73] and interpolated for the room temperature. The error bars indicate the standard deviation calculated from several measurements. (Insert in A) A micrograph of a stainless steel SUS304 microrod in the studied solution. (B) The measured viscosity of aqueous glycerol solutions measured with nickel nanorods versus equilibrium humidity. The tabulated values are taken from Refs. [74] and [75] and interpolated for the room temperature during the experiment. Three probes were used to measure each point. (Insert in B) A micrograph of a nickel nanorod in the studied solution. (C) The temperature dependence of viscosities of the Viscosity Standard S60 and 65% aqueous glycerol solutions. The dotted lines represent the tabulated values of viscosities. The solid symbols represent the data measured with nickel nanorods. Three probes were used for a Viscosity Standard S60 measurement and a single probe was used to probe viscosity of glycerol solution.

To illustrate the capabilities of the humidity control system, the aqueous glycerol solutions of various concentrations were studied by pumping the nitrogen-water vapor mixture through the chamber while keeping the temperature constant. The humidity of nitrogen gas was varied and, simultaneously, the solution viscosity was measured with nickel nanorods. Since the solvent (water) was free to condense or evaporate from the solution, the concentration of water changed in the sample until it reached equilibrium with the humid gas around it. Thus by controlling the humidity around the sample, one controls the water concentration in the sample. Using rotating probes, one can directly relate the equilibrium humidity to viscosity. This is an important advancement for characterization of aqueous droplets and thin films, as it eliminates an unnecessary step of relating both of those parameters to...
concentration – a significant challenge for small samples. The interpolated tabulated values of equilibrium humidity as a function of concentration[74, 76] and viscosity as a function of concentration[75] were used as the references.

The experimental protocol was as follows: nickel nanorods were prepared using electro-chemical template synthesis and characterized in accordance with the protocol outlined in Ref. [34]: the diameter and the magnetization were measured to be 340 ± 30 nm and 160 ± 40 kA/m, accordingly; the length which changes after sonication of the prepared suspension, was extracted from the video analysis. An aqueous solution of glycerol of 50% by weight (79% equilibrium humidity at 22°C) was prepared, in which nickel nanorods were suspended. The humidity in the chamber was then altered to a desired value, thus initiating evaporation of water from the droplet. The critical frequency $\omega_{cr}^{solution}$ was measured as a function of time, until the droplet reached equilibrium. The viscosity of solution at equilibrium was then calculated from the critical frequency and recorded for a given humidity and the process was repeated for a different humidity. The measured values of equilibrium viscosity as a function of humidity of the supplied gas are presented in Figure 9B. Value for the viscosity for 100% humidity was measured my suspending nanorods in deionized water. Again, the experimental points fall on the tabulated curve nicely, confirming the validity of the developed protocol.

To demonstrate the effectiveness of the temperature control, we performed experiments with nickel nanorods in two temperature-dependent viscosity standards – Viscosity Standard S60 (Cannon Instrument Company) and 65% aqueous glycerol solution. To prevent liquid evaporation, all experiments were conducted in a closed container – a glass slide with a cover slip.

The experimental protocol was as follows: the nickel nanorods were suspended in a sample, which was heated to a desired temperature at which the critical frequency $\omega_{cr}^{solution}$ was measured, from which the viscosity was calculated and then correlated with the tabulated value. The sample was then heated to a new temperature, and a new critical frequency was measured and viscosity was calculated. In Figure 9C, the measured viscosities vs temperature of the S60 viscosity standard and the glycerin solution are presented along with the tabulated values of the viscosity standards.

The developed magnetic stage with environmental control system is a convenient tool to study complex solutions undergoing different phase transportations upon solvent evaporation. As an illustration, we show a complex behavior of the viscosity of a surfactant-cosurfactant solution - cetylpyridinium chloride-sodium salicylate-brine (CPCI-NaSal). A phase diagram for this ternary system in the 2D cetylpyridinium chloride-sodium salicylate plane has been characterized structurally as well as rheologically in Refs.[77-82]. These papers dealt with the liquid phases only. The drying kinetics of this system and phase behavior, however, have not been investigated due to the difficulty of tracking of the rheological properties of a drying sample under varying conditions. Here, we present a comparison of the time-dependent microscopic viscosity as well as final morphology of two drop-casted samples of 2% CPCI-NaSal in 0.5% NaCl brine as they dry up in nitrogen of 0% and 57% humidity.

The sample viscosity can depend on the probe location. The viscosity is measured at the location of the probe starting from deposition of the sample until complete encapsulation of the probe by a crystal dendrite. The period of probe rotation ($\tau = 5s$) was chosen to be much greater than the relaxation time of the solution ($\tau = 0.14 \pm 0.01s$)[55]; thus, the Newtonian model of liquid, eq. (4), accurately predicts the
nanorod behavior (Figure 10B) and no elastic contribution is detected. The time dependence of viscosity (Figure 10A) of the two drying samples demonstrates some differences in the drying kinetics.

When the film evaporates, the viscosity of both samples increases until it hits a plateau at relative viscosity of 2. The viscosity of both solutions remain almost constant for some time. This suggests that as both samples evaporate, they reach a phase, in which either evaporation significantly slows down or the viscosity stops being dependent on the concentration of water. The local viscosity of a sample that was dried at 0% humidity, however, gets to the plateau more quickly and increases more sharply several seconds before the crystal consumes the nanorod. Surprisingly, the nanorod does not drift during the dendrite approach until the crystal embraces the nanorod completely.

In contrast to this behavior, the local viscosity of the sample dried at 57% humidity leaves the plateau phase more slowly and takes several minutes to gradually increase its value. During the crystal growth, two nanorods were captured on the video. The nanorod that is positioned closer to the dendrite moves towards it significantly quicker than the one located further away. This suggests that some flow has been developed in the crystal vicinity. The final microstructure of the sample (Figure 10D, left) appears drastically different from that formed at 0% humidity (Figure 10C, left).
Figure 10. (A) The time dependence of viscosity in a thin film of CPCl-NaSal drying in pure nitrogen (0% humidity) and in nitrogen of 57% humidity. The viscosity is measured at the location of the probe starting from the moment of film deposition until the complete encapsulation of the probe by a crystal dendrite. (B) An extracted trajectory along with its fit by eq.(4) for the nanorod in the film held at 57% humidity. To avoid any elastic contribution to the probe rotation, the period of rotation was chosen to be much lower than the relaxation time of the solution. [55] (C left) A polarized micrograph of a sample dried at 0% humidity. A multidomain structure with cracks or grain boundaries is observed. (C right) A gallery of snapshots showing a nanorod in the vicinity of a growing dendrite. The dendrite grows relatively fast and the nanorod does not drift in the sample. (D left) A polarized micrograph of a sample dried at 57% humidity. Multiple dendrites are ordered in arrays. (D right) A gallery of
snapshots showing a nanorod near a growing dendrite. As the crystal grows near the nanorod, the nanorod gets pulled towards the crystal, indicating that the growing crystal generates a flow.

4 CONCLUSION

A multifunctional magnetic stage for optical microscopy with temperature and humidity control was developed. It addresses the challenges associated with magnetic probing of materials at the hundreds of microTesla magnetic fields. Applying a special procedure to zero out a bias ambient magnetic field and apply a uniform rotating field to rotate magnetic probes, the stage was used for analysis of rheological properties of fluids of viscosities ranging from mPa·s to Pa·s with measurements carried out in as little as 10 seconds. To demonstrate the reliability and robustness of the instrument, the rotation of magnetic probes of various sizes has been tracked to study a wide range of solutions with different viscosities in different environmental conditions. By performing viscosity measurements on samples of aqueous glycerol solutions with a free surface in a controlled humidity, it has been demonstrated for the first time that viscosity as a function of humidity can be directly measured without explicitly linking viscosity through concentration of volatile component. The technique also allows one to study kinetics of evaporation and solidification of a model different complex fluids. A surfactant-cosurfactant system (CPCI-NaSal) was used as an illustration of its complex phase behavior at different humidities.

We envision that this multifunctional magnetic stage will be useful not only for the environmentally-controlled nano and microrheological studies, but for various applications where optical tracking of response of material or probes to low magnetic fields is critical.

SUPPLEMENTARY MATERIAL

See supplementary material 1 for a description of the supply system for nitrogen gas - water vapor mixture.

See supplementary material 2 for details on theoretical and experimental determination of the lower bound of the flow rate in the environmental chamber.

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