Chapter 2
Feedback Trap

The feedback trap is a device for trapping and manipulating single particles in solution [1]. The main advantage of this device is its ability to trap molecules and other small objects directly, rather than attaching them to a micron-sized bead or encapsulating them. The feedback trap counteracts the random thermal fluctuations that perturb the motion of small objects in a finite-temperature fluid. The basic idea is to periodically measure the position of an object and then to calculate and apply a force to keep the particle in the field of view and manipulate it. In contrast to optical tweezers, there is no physical potential to trap the particle; rather, the action of the feedback loop creates a “virtual potential” that can confine a particle or force it to perform more complicated motion. Absent the feedback loop, there is no potential and the particle diffuses freely in a fluid cell.

Feedback traps have been used to measure physical and chemical properties of particles and molecules [2–9] and to explore fundamental questions in the non-equilibrium statistical mechanics of small systems [1, 3, 10–17]. In my research, I focused on the latter applications, where I impose a trapping potential \( U(x, y, t) \), to explore particle dynamics. The idea of imposing a virtual potential was originally proposed by Cohen, who designed the first feedback trap. He studied the motion of a particle in a virtual double-well potential and in potentials of the form \( U(r) \sim r^n \)[3]. I have used time-dependent virtual potentials to study the Landauer Principle [12, 18, 19], which relates information erasure to thermodynamic work, and I used the trap for many other precise measurements in stochastic thermodynamics.

The major issue with the feedback trap is to understand carefully the motion it induces in particles. For example, it is clear that imposing a force that is the gradient of a potential \( U(r) \) is not quite the same as imposing a physical potential. In a physical potential, the force adjusts continuously as the particle moves. In a virtual potential, the force is updated after a time \( t_s \). Thus, we expect that the motion to be at best a discrete approximation to the true potential.

Several attempts have been made to quantify the dynamics of a feedback trap. The complications due to a finite time \( t_s \) between updates were resolved earlier [20, 21],
but Jun and Bechhoefer [10] have taken into account other considerations, including finite camera exposures and the associated delays.¹

In my initial work, I mostly focused on experimental challenges of imposing an arbitrary potential and applying an electric force on a particle. In this chapter, I will present experimental setup of a feedback trap that I used for various tests in stochastic thermodynamics. In the following chapter, I show the developed calibration method which estimates, with high precision, a particle’s properties and uses them for accurate control of the imposed potential.

2.1 Contributions to This Chapter

The content of this chapter has been published in two papers. The details about the fluorescence-based feedback trap are in SPIE Proceedings [11]. This version of a feedback trap was built by Yonggun Jun. I added an acousto-optic laser deflector to control the particle bleaching rate and increase its lifetime, and I designed and fabricated microfluidic chips in 4D labs at SFU (see Appendix A). John Bechhoefer and I wrote the article.

Jan Koloczek, an undergraduate summer student, worked on different non-fluorescence particle-imaging techniques. His exploration of different darkfield-illumination methods led me to the current illumination design described in Ref. [14]. John Bechhoefer and I wrote the article. Later, I found an alternative, more affordable, and simpler method to replace the microfluidics.

2.2 Experimental Setup

I use a feedback trap to confine motion of a micron-size bead in a potential of arbitrary form, to explore different questions in stochastic thermodynamics and information theory. In the initial version of a feedback trap, which I inherited from a postdoc Yonggun Jun, I trapped fluorescent beads that were 210 nm in diameter [11]. Later, I developed a new setup which uses scattering-based illumination to trap silica beads of 1.5 μm in diameter [14]. Although two-dimensional feedback trapping of both types and sizes of beads works in the same way, the passive trapping in z-direction is quite different. Smaller beads are confined in a micron-size channel, while larger beads sink down because of gravity.

¹Some versions of the feedback trap use a single detector to time photon counts from a rapidly scanned laser beam. For such systems, there is no exposure time, although there continues to be a lag between the photon detection and the response. However, while the effectively instantaneous detection of photons and consequent lack of a finite camera exposure time simplifies some aspects of the dynamics, the Poisson statistics associated with both signal and background and the need to use sophisticated filtering techniques create difficulties of their own [22, 23].
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A feedback trap confines motion in a virtual potential in the $xy$ plane. Initially, the desired trapping potential is specified. The potential could be either static or it could change with the time. The virtual potential is created by rapidly cycling through three steps:

- observe the position $(\bar{x}_n, \bar{y}_n)$ of a trapped object;
- calculate a force from the imposed potential at the observed position $\bar{F}_n = -\nabla U|_{\bar{x}_n, \bar{y}_n, n, \Delta t}$;
- apply a voltage proportional to the calculated 2D force to four electrodes that create a horizontal field in the $xy$-plane.

The field is kept constant until updated with information acquired from the next image. For my implementation of a feedback trap, the position is observed in two steps, by first acquiring an image and then using a centroid algorithm to estimate the position [24]. Figure 2.1 shows the operation of a feedback trap with these steps separated explicitly. The EMCCD camera (Andor iXon DV-885) attached to a microscope takes an image of particle during some finite exposure time $t_c$. The image is sent to a PC, and the LabVIEW code estimates the centroid of an image after thresholding. This centroid gives the observed position $(\bar{x}_n, \bar{y}_n)$, which differs from the actual position of a particle $(x_n, y_n)$. From the imposed potential $U(x, y, t)$ and the observed positions, a computer calculates forces and generates voltages that are sent to the data acquisition device (DAQ), amplified $15 \times$ through a home-built amplifier, and sent to the electrodes. The delay between observation of a position and application of voltage is set to be equal to the update time of the feedback trap $t_d = t_s$.

The step of generating and calculating voltages from given forces is the trickiest step, and Chap. 3 on calibration is dedicated to explaining that process. In addition to trapping and collecting data, the setup also stabilizes the intensity of light coming from the trapped bead to keep the observation noise in the desired range. It also

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Fig. 2.1 Schematic of feedback trap operation. a Acquisition of an image of a fluorescent particle. b Determination of particle position from that image using a centroid algorithm. c Evaluation of feedback force $F_x = -\partial_x U(x, t)$ at the observed position $\bar{x}$. d Application of electric force, with voltage set by electrodes (light blue), held constant during the update time $t_c$. The long gray arrow indicates repetition of the cycle.
adjusts the focus, so that the bead is always visible, calibrates the system recursively, and monitors for potential errors and various unwanted events (See Sect. 3.9.2).

### 2.2.1 Fluorescence-Based Imaging Feedback Trap

Our initial experimental setup broadly follows that of Cohen and Moerner [25, 26], with some adaptations and simplifications. Below, I describe the overall setup and sample cell, the illumination, the image capture and processing to extract particle position, and the application of electrical forces.

The setup is designed around a home-built epifluorescence microscope made from standard optomechanical parts and incorporating a 100X, NA=1.25 oil-immersion objective (Carl Zeiss Jena, Achromat HI100x). The general setup is illustrated in Fig. 2.2. An 800-nm-thick flow cell was fabricated by spincoating a layer of photore sist (SU-8) on a glass surface. The SU-8 is used as a spacer rather than as simply a mask for etching. To bond the SU-8, we heat the sample to 180 °C and firmly press the cover glass onto the SU8-coated base plate [27]. The base plate was first drilled with 1-mm glass holes for the electrodes. The detailed protocol for the fabrication of the flow cell is presented in Appendix A. Compared to the cells used by Cohen and Moerner, [25, 26] the present design is simpler, as there is no etching of the glass. One small difference is that in our design, the triangle-shaped areas in Fig. 2.2 are

![Fig. 2.2](image_url)

**Fig. 2.2** Schematic of experimental setup. a Microfluidic cell design. The trapping area (blue) is sealed by SU-8 photoresist (gray), which also serves as an 800-nm-thick spacer. b Plexiglas sample holder (yellow) stores approximately 600 ml of water solution with beads in four wells (blue). One electrode (black) is placed in each well. c Apparatus: A camera takes images of particles in an inverted epifluorescence microscope based on a 532-nm laser. The computer sends output voltages via the DAQ to the electrodes, closing the feedback loop. POL denotes a linear polarizer, AOD an analog acousto-optic deflector, and LP denotes two long-pass filters that block residual laser reflections and transmit fluorescent light at wavelengths > 565 nm
800 nm thick (and not 20 μm, as in Ref. [26]); as a result, there is a greater voltage drop before reaching the central experimental chamber (the square 1 × 1 mm region in Fig. 2.2). However, in our experiments, we were not limited by field strengths.

We use a 5-mW, 532-nm laser (Z-Bolt, DPSS-5M) for illuminating particles. Under laser illumination, particles photobleach. To maximize the observation time, we minimize the illumination area so that particles outside the field of view are not illuminated unnecessarily. We set the illumination area by using an analog acousto-optic deflector, or AOD (AA Opto-Electronic, Model DTSXY-250-532), to rapidly scan a tightly focused laser beam at a rate of 100 kHz (x-axis) and 61.8 kHz (y-axis). The frequency ratio is chosen to be near the golden ratio, \( \frac{1}{2} (1 - \sqrt{5}) \approx 0.618 \), which is the number that is “closest” to an irrational number in the sense that any finite approximation will have a high rational denominator. The resulting Lissajous figure for the beam thus covers relatively evenly the entire field of view [28]. The AOD has the important side benefit that moving the beam rapidly averages out speckle, improving the homogeneity of the illumination. We image fluorescent polystyrene divinylbenzene particles that are 210 nm in diameter (Thermo Scientific, R200 Fluoro-Max Fluorescent Particles, dyed red) in deionized water.

A further significant advantage of the AOD is that we can alter the intensity to compensate for the photobleaching of the fluorescent particle [29]. Since the observational noise \( \chi \) depends on the particle’s fluorescent intensity, regulating that intensity meant that the noise statistics did not drift with time. To stabilize the observed fluorescent intensity, we first time-averaged the particle intensity signal using a running-average filter with time constant 2 s. The intensity fluctuations mostly result from the vertical motion of the 200 nm particle in the 800 nm trap. As the particle diffuses up and down vertically, it samples a laser beam whose intensity varies. We fed the time-averaged particle position into a proportional-integral control algorithm that adjusted the AOD intensity, which in turn regulated the intensity of the deflected laser beam. Figure 2.3 shows time traces of the laser illumination intensity and the observed fluorescence. The typical lifetime is about two hours, which was long enough that we could measure the effect of feedback gain variations on a single particle.

We use an electron-multiplying CCD (EMCCD) camera (Andor iXon DV-885) to acquire 100 × 100 pixel (8 × 8 μm) images at 100 Hz, with an exposure time \( t_c = 10 \text{ ms} \) and 2 × 2 pixel binning. We recall that the control program must not only acquire images but must also, for each image acquired, find the position of the particle, compute a response, and output the appropriate voltages to the electrodes.

The camera exposure (and overall experiment) is controlled via a program written in LabVIEW (2009), with the image-analysis routines written in Mathscript (a Matlab-compatible language). To estimate the particle position, we use a simplified version of the centroid algorithm of Berglund et al. [24]. In our version, we first estimate the position of the particle by locating the pixel of maximum intensity. We then center a region-of-interest box around the first estimate and use the centroid algorithm to make a more precise estimate. Using an approximately centered region of interest reduces to negligible values the bias discussed in Ref. [24].

After estimating the position, the algorithm calculates the displacement of the particle from its desired position and applies the electric field needed to bring the
particle to the desired position during the interval $t_s$. The force is created by applying voltages across two pairs of Pt80/Ir20 electrodes (0.25 mm in diameter, Goodfellow Corp.), as depicted in Fig. 2.2. The delay time between estimating the position and applying the force is set to $t_d = 10$ ms.

### 2.2.1.1 Hardware Properties of a Feedback Trap

We need to characterize the various hardware parameters that affect the observed particle dynamics, which include observational noise and timing. The observational noise $\chi$ depends on the microscope point spread function, camera pixel size, camera read and background noise, image background variations, and the intensity of the fluorescent light emitted by particle. We characterized its magnitude by immobilizing a 200-nm fluorescent bead on a glass surface and repeatedly measuring its position. Regulating the incident laser intensity $I_L$ as shown in Fig. 2.3, we measured the standard deviation as a function of the observed mean fluorescence intensity. The result, Fig. 2.4, approximately follows the expected $I^{-0.5}$ power law expected for shot noise, with excess noise at higher intensities. We typically regulated the fluorescence intensity to a level of 1000, which corresponds to $\chi \approx 22$ nm and a particle “lifetime” of about two hours, before it bleaches. These values are a compromise, in that maximizing $I_L$ reduces observational noise, while minimizing $I_L$ increases

**Fig. 2.3** Proportional-integral control of the fluorescence light intensity. The decrease in fluorescence intensity due to photobleaching was compensated by increasing illumination laser intensity. **a** Illumination laser intensity increases with time up to the saturation point. **b** The intensity of fluorescence light (red lines) is kept constant by the AOD. Black solid line represents the intensity averaged over 20 seconds. After the AOD saturates, the intensity decreases. All intensity values are given in arbitrary units.
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Fig. 2.4 Observational noise $\chi$ as a function of fluorescent light intensity. The red markers are measurements on immobilized beads. The black solid line is a fit to the $\chi \sim I^{-0.5}$ power law expected for shot-noise-limited images. The dashed line indicates typical operating conditions.

The timing parameters were determined by temporarily adding an LED light between the LP filter and the camera (Fig. 2.2). Using the digital output from a data acquisition card (DAQ), we generated a $\tau = 0.1$ ms LED light pulse each 60 ms, as verified using a photodiode. The DAQ’s analog output was set to be proportional to the light intensity detected by the camera. The analog output, camera trigger, and $\tau$ pulse were all measured by an independent DAQ and computer, sampling at 1 kHz. By adjusting the phase of the $\tau$ pulse relative to the camera trigger, we could make the pulse straddle the end of one exposure and the beginning of the next. Since this was possible with a 0.1 ms light pulse, we conclude that the “dark time” between end of one exposure and the beginning of the next was less than this time, which is short compared to the 10 ms exposure time. Thus, we conclude that the exposure time is close to the update time $t_c = t_s = 10$ ms. The response delay $t_d$ is evaluated by measuring the time difference between the $\tau$ pulse straddling two exposures and the time that the DAQ voltage is updated. We set the DAQ update so that $t_d = t_s$. One potential ambiguity is that a delay of an integer multiple of $t_s$ could appear (as in aliasing) to be a shorter, fractional delay. We resolved this potential ambiguity by sending a pulse every 60 ms, or 6 time steps.

The first version of a feedback trap, based on fluorescent imaging of 200-nm beads was used for developing a calibration routine [30] and for testing Landauer’s principle [12].

2.2.2 Darkfield-Based-Illumination Feedback Trap

The initial version of the feedback trap relied on fluorescence microscopy for estimating particle positions. This technique causes particles to photobleach, which limits their lifetime in the trap to just a few hours [11]. After a particle has photobleached,
the feedback trap searches for a new particle, recalibrates, and continues trapping. As described in Chap. 3, the process of searching for a new particle and recalibrating is slow, and repeating it every few hours decreases the productivity of a trap. Fluorescence microscopy also outputs low light intensities, forcing us to use long exposure times and high gain on a sophisticated EMCCD detector.

Scattering-based illumination can potentially give much higher photon count rates than fluorescence microscopy [31]. The photon count is effectively unlimited, in contrast to fluorescence, where the count rate is limited by the physical properties of the fluorophore (saturation, bleaching). As a result, a particle imaged using scattering-based illumination can in principle be trapped indefinitely. This illumination approach is especially suitable for measurements in stochastic thermodynamics, where a large amount of statistics can be collected using a single bead. The high photon count rate allows one to use new classes of light detectors, where speed does not have to be compromised for sensitivity or, in our case, turn off the amplifier gain, use shorter exposure time and run the camera at a higher bandwidth. The important property of scattering-based illumination is the signal-to-noise ratio. A high signal-to-noise ratio is achieved by illuminating particles with a strong incident beam, efficiently collecting the scattered light, and blocking the incident beam. Blocking the incident beam needs to be done using geometry, in contrast to the filters used in fluorescence illumination. In the context of microscopy, the geometry-based removal of incident light is known as dark-field microscopy.

2.2.2.1 Dark-Field Microscopy

Dark-field microscopy is traditionally implemented using glancing-angle illumination by a high numerical aperture (NA) condenser and collecting rays with a lower-NA objective [32]. The low-angle rays are blocked before the condenser, so that only a cone of illumination light is used.

With the help of an undergrad student, Jan Koloczek, we implemented a first version of this, based on a design by Lebel et al. [33]. This design gave a high signal-to-noise ratio and could trap a particle for long time. Figure 2.5a shows our implementation of a feedback trap using this traditional form of dark-field illumination. The disadvantage of this setup was that it led to an asymmetric point-spread function, caused by the asymmetry of the illumination [34]. Real-time calibration, a necessary step for precision measurements, is difficult to implement with radially-asymmetric point-spread functions [30].

Because of these difficulties, we have implemented a “reverse dark field” configuration instead, following work of Weigel et al. [31]. We use a low-NA condenser and a high-NA objective with a beam blocker. (See Fig. 2.5b) The illumination system, together with the circular beam blocker, makes the point-spread function radially symmetric, which improves estimates of system properties (particle diffusion, mobility, and observational noise) [30]. It also uses the illumination power efficiently.

The system shown in Fig. 2.5b is very simple to implement and uses standard optics, but it requires very precise and stable alignment. The Rayleigh range (depth
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(a)

Fig. 2.5 Scattering-based dark-field illumination designs. a Conventional approach using back scattering: The laser beam (red) is guided through the edge of a high-NA objective using a 45° mirror. Trapped beads scatter light (pale red), which is collected in the central area of the objective and focused onto the camera. b Reverse dark-field design: The LED light beam (dark red) is focused on beads in water. The parallel beam is blocked at the back of the objective; only high-angle forward-scattered light (pale red) from the particle is focused onto the camera.

of field) in our system is much greater than the vertical confinement area; as a result, the intensity of scattered light is insensitive to vertical motions of the bead.

2.2.2.2 Comparison Between Fluorescence and Darkfield-Based Imaging

Initially, we imaged 200-nm beads using two different methods: a home-built fluorescence microscope (see Fig. 2.2) and the reverse dark-field microscope presented in Fig. 2.5. With fluorescence, the 200-nm bead was visible for only two hours, while the same bead could be observed for a very long time using scattering. The mean photon count rate was 6000 times greater when the reverse dark-field microscope was used under similar conditions. This significant increase in intensities allows us to decrease the exposure time and simplify the feedback trap theory used for real-time calibration (see Chap. 3). Unlike in fluorescence, where the count rate is limited by finite number of fluorophores, dark-field illumination does not set an upper limit on the count rate. This allowed us to use illumination sources with greater power and decrease the exposure time. Potentially, the camera can be replaced by a position-
sensitive detector, whose bandwidth can easily reach 10–100 kHz, which is more than 100 times the bandwidth of our present trap.

2.2.3 Working Particles and Latest Experimental Setup

The size of the polystyrene beads was used initially 210 nm in diameter. They required confinement in an 800-nm-thick channel made of SU-8 photoresist to keep them in focus. During the SU-8 fabrication process, some portion of photoresist remains on the nominally clean area. Such ‘stains’ on the surface also scatter light, making the background nonuniform and the estimation of a bead position more challenging.

To overcome these issues, I used 1.5-μm (in diameter) silica beads. These beads are large enough and heavy enough that gravity confines their motion to within the depth of focus of our microscope, yet small enough to diffuse freely in the horizontal plane. The particles are made of silica with density $\rho_s = 2.2 \text{ gm/cc}$ and diluted in water. This approach required no confinement in the vertical direction and no need for sophisticated micro-channel fabrication. Using larger beads results in an overall

![Fig. 2.6](image)

**Fig. 2.6** Complete scattering-based illumination feedback trap. a LED light source illuminates the trapped silica bead. b Bead in sample cell sinks under gravity and diffuses predominantly in the XY plane. c High-NA microscope objective collects light scattered from bead and also directly from LED source. d Beam blocker stops the LED beam, allowing only scattered light from bead to reach the camera. e Camera takes image. f Image-processing program estimates bead position and g calculates the force based on the imposed potential. h DAQ applies voltage proportional to the calculated force to electrodes. i Forces due to electric field and thermal fluctuations move bead to a new position. The feedback loop repeats indefinitely, with cycle time $\Delta t = 5 \text{ ms}$
better signal-to-noise ratio, because larger beads scatter more light relative to the background.

Finally, in Fig. 2.6, I show the complete scheme of the latest scattering-based-illumination feedback trap.

References

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Experiments on the Thermodynamics of Information Processing
Gavrilo, M.
2017, XVI, 147 p. 55 illus., 53 illus. in color., Hardcover
ISBN: 978-3-319-63693-1