Lab Course Experiment

Microfluidics Driven By Light

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A long term goal is the direct optical control of biomolecules and water for applications ranging from microfluidics, over biomolecule detection to non-equilibrium biophysics. To reach this goal, thermal forces originating from optically applied, dynamic microscale temperature gradients have shown great potential.

This experiment investigates a technique to generate and control arbitrary flow pattern in any thin liquid layer by the repetitive movement of a focused infrared laser.

I. Light Driven Microfluidics

Basic Principle.

A focused infrared laser is used to generate temperature changes in micrometer thin water layers (Fig. I.1a). Due to fast thermal coupling to the near glass windows, temperature spots can be generated with diameters of only a few micrometers, whose intensity and position can be changed with rates faster than 10 kHz. As result, the water in the wake of the spot cools down very fast, when the heating laser spot is moved through the layer. For moderate velocities, the result is a moving warm spot. The water in front of the spot heats up while the water in the wake of the spot cools down to ambient temperature again. The resulting density changes of the water lead to divergent flows due to mass conservation [I.1]. In front of the spot the water expands, while in the wake it contracts again. At constant viscosity the system is symmetric and the contraction exactly cancel out the previous expansion. However, including the temperature dependence of the viscosity breaks the symmetry. A lower viscosity at higher temperatures for example increases the velocities in the warm spot between the expansion and contraction (see bottom of Fig I.1b). Result is a net flow against the spot movement direction. When the spot passed over once, the net shift of the water after expansion and successive contraction is in the tens of nanometer scale. However, the laser movement can be repeated in the kHz regime due to the fast thermal coupling to the close glass windows. Therefore reasonable fast pump velocities in the order of 100 µm/s can be achieved, generated and defined by the movement of the laser.

Using the nonlinear effect described here, we can, for example, move water along the letters “LASER PUMP” without lateral walls to guide the flow (Fig. I.2). In this example, we sandwich a 10µm thin sheet of water between glass slides and move a focused infrared laser along the letters. The water is locally heated by direct absorption of the laser irradiation. The spot movement along the letters is repeated in the kilohertz regime. As a result, the fluid flows the reverse path of the warm spot movement.

Finite Element Simulations and Theory

To test the model of thermally induced asymmetric expansion, a finite element simulation of the three dimensional Navier-Stokes equation was performed. The system of interest allows to do a set of approximations. We can for example assume the flow to be laminar and therefore neglect the inertia terms. Furthermore

dimensions parallel to the surface are much greater than the width of the water layer, which allows to do a thin film approximation and describe the system as a two-dimensional flow. By further approximation, which can be tested by the finite element simulations (for details see [I.2,I.3]), it is possible to obtain an analytical formula, which describes the pump velocity under a set of parameters:

$$v_{\mathrm{pump}} = -\frac{3\sqrt{\pi}}{4} f \alpha \beta b \Delta T^2 , \qquad \text{(I.1)}$$

where f is the repetition frequency of the laser, α is the expansion coefficient, β is the temperature dependence of the viscosity and ΔT is the amplitude of the temperature in the spot. The temperature can be measured by imaging a temperature sensitive fluorescent dye added to the water. The shape of the moving warm spot is measured by stroboscopic illumination with a LED with a 10 µs long rectangular light pulse each time the laser spot passes by. The parameter b is the width of the heat spot.

Since all parameters used in (I.1) can be measured, the theoretical formula can be tested without fitting parameters. We have argued that every passage of the warm spot results in a liquid step Δx opposite to the spot movement direction. The pump speed is therefore expected to increase in a linear fashion with repetition frequency of the laser spot as given by equation (I.1). This predicted behavior can be verified by measurements in the low frequency regime $f \ll 1\,\mathrm{kHz}$ as shown in the inset of figure I.3a. The repetition rate f in this experiment was adjusted by moving the laser spot with increased velocity along a fixed circular pump geometry. For slow frequencies, the spot temperature distribution remains remains roughly radialy symmetric, with a Lorentz-Cauchy distribution. However, a further increase in the spot velocity results in a considerable elongated temperature spot geometry along the pump direction which enhances the pump speed beyond the linear estimate for constant spot width b (Fig. I.3a). Examples of spot geometries as measured with stroboscopic temperature imaging are given as color coded insets. Interestingly, the enhanced pump velocity at high laser spot velocities could still be described when the elongated temperature spot geometry is taken into account with a spatial integral of equation (I.1) for each repetition rate (Fig. I.3a, solid line).

The analytical theory predicts a linear response of the pump velocity to both the thermal expansion $\alpha \Delta T_0$ and the change in the temperature dependent viscosity $\beta \Delta T_0$ for a similar shape of the temperature spot. If the spot temperature is enhanced by higher laser power, the pump velocity increases proportionally to ΔT_0^2. The experiments fully confirm this parabolic dependence as shown in figure I.3b. The solid line results from equation (I.1) without additional fitting parameters and matches the experimental data within the error bars.

The proportionality to the parameters α and β are tested by changing the ambient temperature T_{ch} of the chamber. In figure I.3c we changed T_{ch} by cooling the microscope stage with an external heat bath. The experiments reveal a reversal of the pump direction at fluid temperatures below 4 °C. We attribute this reversal to the sign change of the volume expansion coefficient α. The theoretical expectation of equation (I.1) again fits the experimental pump velocities as plotted against the fluid temperature within the experimental errors.

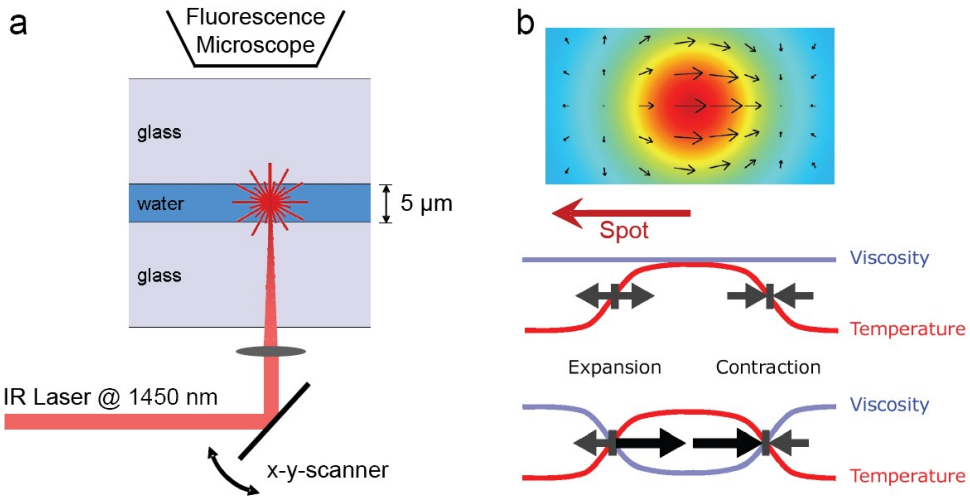


Figure I.1: (a) Schematic view of the experimental setup. An infrared laser is deflected by a Acousto-Optical Deflector and focused into a thin water layer between two glass windows. The water absorbs a part of the light. Result is a spot of raised temperature localized at the optical focus of the laser. (b) Moving the warm spot generates local fluid flows due to density changes of the water. The water expands in the front of the spot and contract again in the wake. At constant viscosity both movements cancel out one another. However, the lower viscosity of the liquid at higher temperatures increases the velocities inside the warm spot. Result is a net flow against the spot direction.

II. EXPERIMENTAL SETUP

For this experiment we use a standard fluorescence microscope combined with an infrared laser scanning capability.

The sample consists of a very thin sheet of liquid sandwiched between a glass slide and a glass cover slip.

For heating a fiber laser with a wavelength of 1455 nm is focused into the water

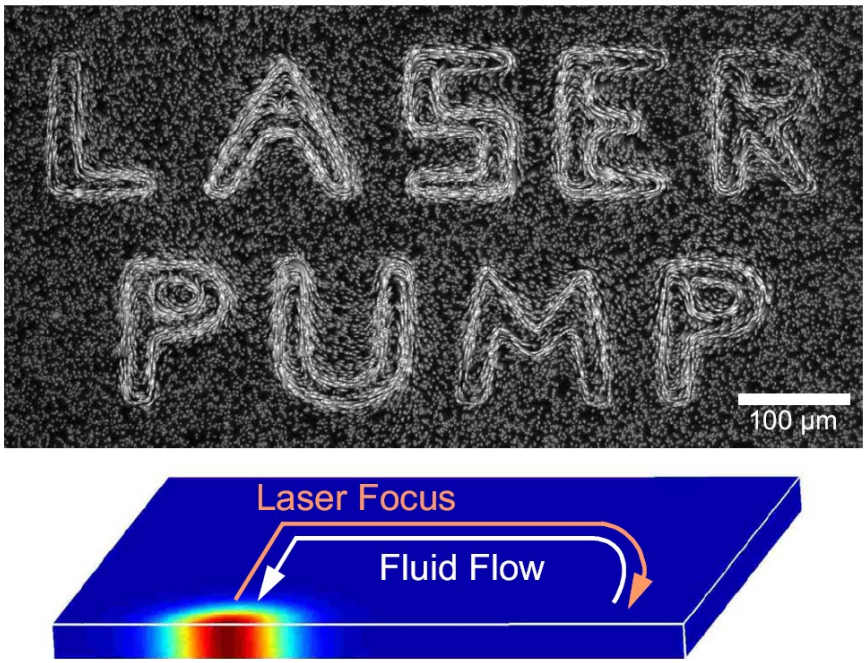


Figure I.2: Pumping water optically along arbitrary patterns. Fluid flow along the letters "LASER PUMP" is driven by dynamically heating a thin fluid film with a laser scanning microscope. As seen, complex flow patterns are easily accomplished. No channels restrict the fluid flow. Local pumping of the fluid film is the result of thermoviscous fluid movements for each passage of the laser focus. We visualize the water flow by fluorescent tracer particles.

layer. Water absorbs this wavelength strongly with an attenuation length of 305 µm.

III. Fluorescence

Introduction

In many applications in science it is the mayor task to observe **only one or some** components of complex systems such as cells or ensembles of molecules. Biology and biophysics research is nowadays concentrated on the size scale of micrometers to nanometers, which means on length scales of cells down to single proteins.

A very common way to fulfill this task is to mark molecules with fluorescent dyes and to observe the emitted light of these molecules.

Very nice recommendable tutorials about fluorescence can be found on: http://www.invitrogen.com/site/us/en/home/support/Tutorials.html

To understand how fluorescence work, two aspects have to be taken in consideration: The fluorescent dye and the fluorescence microscope.

How does a fluorescent dye work?

Fluorescent dyes are organic or inorganic molecules with a metastable energy level. They absorb light of a certain wavelength, the so called absorption wavelength, relax to a lower, metastable energy level (which means it has a longer residue time than statistically expected) from which they further relax to the ground level by emitting a photon of a slightly longer wavelength. In Figure 2 an energy diagram and the typical spectrum of the fluorescent dye Cy5 are shown. The latter one is a screenshot from the '*invitrogen spectra viewer*', which is a very useful online tool provided by a supplier of dyes to find the right dye-filter combination. Its free, you find it here:

http://probes.invitrogen.com/servlets/spectraviewer

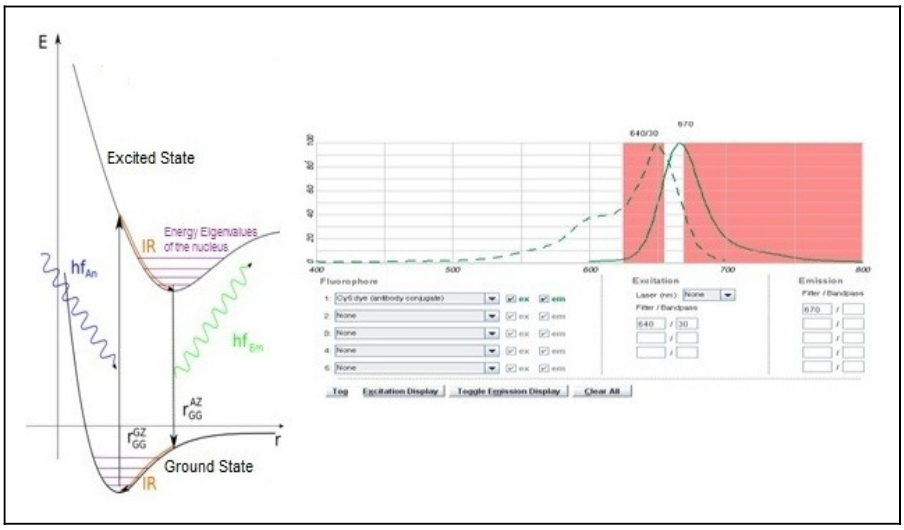


Figure 2: Left: Excitation and Emission of a fluorophore;

Right: Excitation- and Emissionspectra of Cy5 (Red areas mark the transmission wavelengths of the filter)

To understand the process in more detail a quantum mechanical description is required. The Schrödinger equation for the electron wave functions of a molecule provides the potential for the movement of the nucleus which tends to the minimum in such an energy landscape.

If energy in form of photons with the adequate wavelength is applied to the electronic system it is lifted into the excited state. Subsequently it loses energy due to rotations or oscillations in order to reach the new energy minimum. This new state has still a higher energy than the ground state, but also a slightly lower energy than directly after the excitation. To reach the ground state again the system emits a photon with the wavelength corresponding to the remaining energy gap. Therefore the emission wavelength is slightly longer than the absorption wavelength.

In the course of time the ability of fluorescence of the dyes decreases. This is caused by an effect called photo bleaching. In this process the photon energy changes the potential energy of the electrons (e.g. isomeric transitions) instead of inducing the emission of another photon. Thus the energy eigenvalues of the system change and it is no longer excitable with the applied absorption light. Subsequently the fluorophores are afflicted with permanent bleaching.

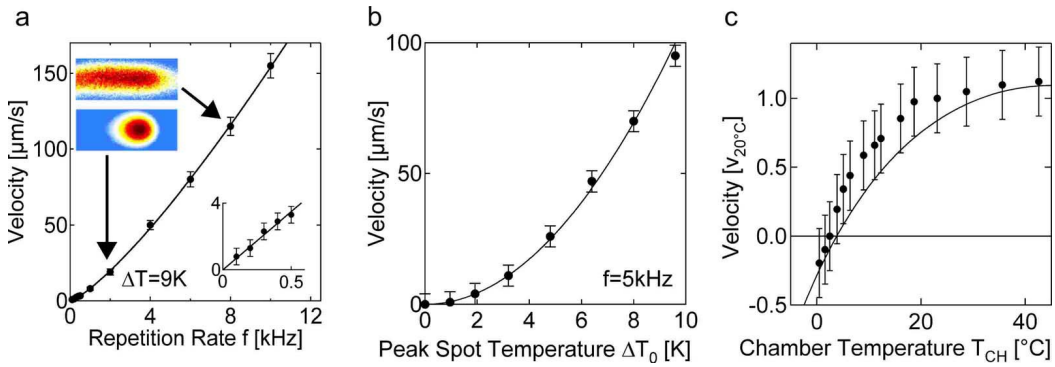
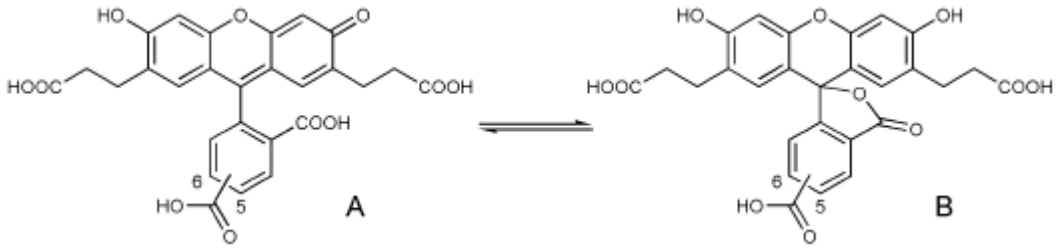


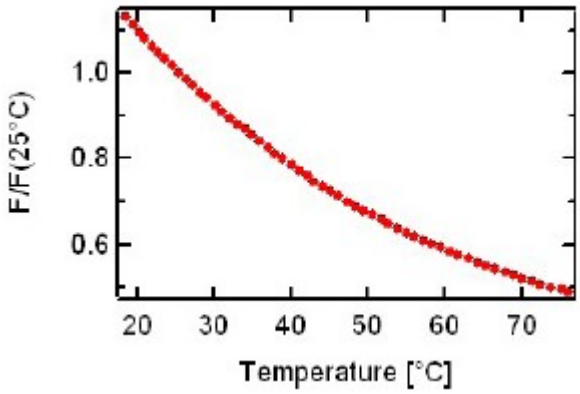
Figure I.3: Testing and comparing the analytical formula with measured pump velocities. (a) The pump velocity is a linear function of the repetition rate for f=1 kHz when the spot geometry remains Gaussian. (inset: temperature image). At faster rates, the warm spot becomes elongated due to the finite thermal equilibration time of cooling. Accordingly, the pump velocity is enhanced beyond the linear prediction as the spot width b increases from 10 to 20 µm in the 5 µm thin fluid film. The solid line predicts the pump velocities based on extrapolated temperature profiles for each repetition rate f. (b) The pump velocity rises with the square of the spot temperature, confirming the linear dependence on both the thermal expansion and the temperature dependence of the viscosity (Fig. I.3b). Pump velocities are predicted by Eq. (I.1) without fitting parameters at a spot width b=25 µm. (c) By changing the overall chamber temperature T_{ch}, we can probe the dependence on $\alpha(T_{ch})$ and $\beta(T_{ch})$. For $T_{CH}<4\,°C$, the water contracts upon heating. As expected from Eq. (I.1), pump velocity reverses its direction (solid line). In all plots, error bars show standard errors from particle tracking.

Temperature dependence of fluorescent dyes

The temperature dependence of the fluorescence dye is determined in independent measurements with 2',7'-bis-(carboxyethyl)-5(6)-carboxyuorescein (BCECF), that serves as a pH-sensitive indicator in classical microscopy.



The structural isomerism of A and B are keto enol tautomerizations, that are present in physiological (pH = 7.4) conditions. The thermal dependence of BCECF is illustrated below, whereas the relative fluorescence is normalized to 1 at 25° C.



An increase in the temperature of 1K displaces the pH-value of the TRIS-buffer instantly about approximately -0.03 pH units. The BCECF fluorescence decreases due to fast proton transitions linearly with moderate thermal enhancements of about -1.46%/K. By measuring the percentage drop of the relative fluorescence in the laser heat spot, we extract its caused temperature elevation in the solution.

As soon as one knows the temperature dependence of the dye, one can use fluorescence as a thermometer. With this technique, we will measure the temperature of the heat spot.

Fluorescence Microscope

*) Question 4: How does a normal light microscope work? What components do you need at least? Please be able to make a small drawing of all lenses and light path.

The setup of a fluorescence microscope resembles a normal light microscope. However, the objective is infinity corrected, which means that the light is parallelized between the tubus lens and the objective. This facilitates the insertion of optical devices into the optical path without influencing the imaging. A typical setup is depicted in the left part of Figure 3: The excitation light is filtered from a LED light source and directed onto the sample via a dichroic mirror. The special characteristic of this mirror is to split up absorption and emission light of fluorescent dyes. It allows the transmission of the emission light, while it reflects the excitation light. This feature is displayed in the spectrum in figure 3. An additional emission filter in front of the tubus lens finally reflects all remaining diffuse light to ensure that only the emission light gets to the camera. The insertion of so many filters reduces the noise, which is immensely important for biophysical applications.

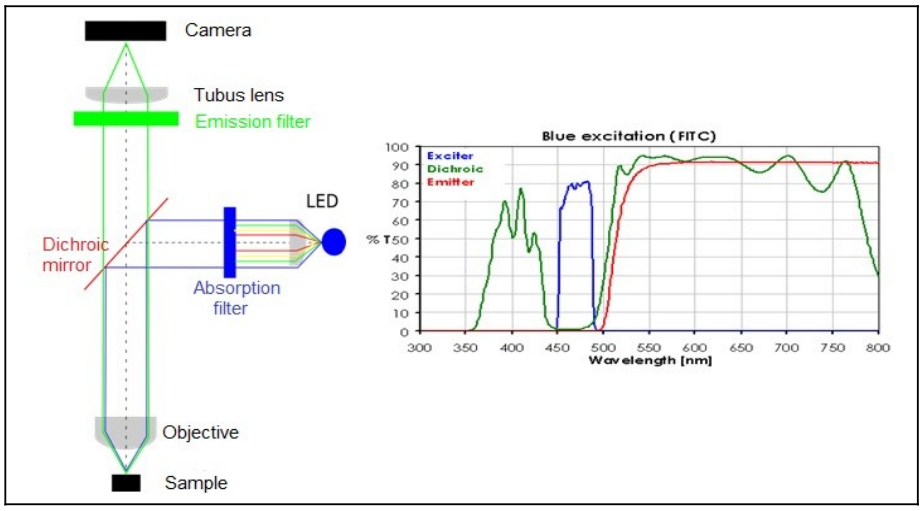


Figure 3: Left: Optical path of a typical fluorescence microscope; Right: Characteristics of a dichroic mirror

***) Question 5: In our lab, we use fluorescence microscopes which allow to observe two different colors at the same time. What additional components do you need for that?

Light sources and lasers

For fluorescence microscopy, light sources with high intensities and a well defined color spectrum are highly needed. Specialized light sources are used such as halogen lamps, steam lamps with several metal steams (Hg, Na) LED´s in different colors and intensities and lasers. In our setup, we use a LED for illumination and an infrared laser to establish the temperature spot.

Therefore it is necessary to lead your attention to the dangers the work with lasers in general and invisible laser beams in special brings with it.

1. Never look into the laser directly or indirectly. Use a laser card to make the IR Laser invisible
2. Before you switch on anything, consider the full way of the laser beam. Make clear where you probably could get in contact with the laser.
3. Make sure that there are no reflective parts around the laser beam (like aluminum, adornment, rings ect.)
4. The laser is guided into the instrument via a light fiber that looks like a yellow cable. The fiber is intrinsic part of the laser, as soon it gets folded or damaged, the laser is gone.

In general: the laser beam of the instrument you use is completely closed, as long as you don´t disassemble something, you are pretty save. Nevertheless it is important that you are aware of the laser.

*) Question 6: Describe the basic principle of a laser.

Hint: Use the term “stimulated emission, the description is oral and should not last longer than three minutes.

To control the position of the laser beam, an acousto-optical deflector (AOD) is employed. This device controls the angle and intensity of the laser beam. The laser light is diffracted by density pattern due to standing acoustic waves in a transparent crystal. By tuning the frequency and the amplitude of these acoustic waves both the angle and the intensity of the used laser can be controlled.

Since this measurement gives results for every pixel from our fluorescence image, we can in fact see the shape of the heat distribution.

Without a confocal setup, the Temperature information will be an average over the temperature of the dye from the buttom to the ceiling of our liquid chamber.

IV. Fluid Flow

As the fluid flow is induced into the liquid, the motion of suspended fluorescent beads can be recorded.

Our camera saves a series of images. The frame rate needs to be noted, since it is necessary for interpreting the the speed of the beads.

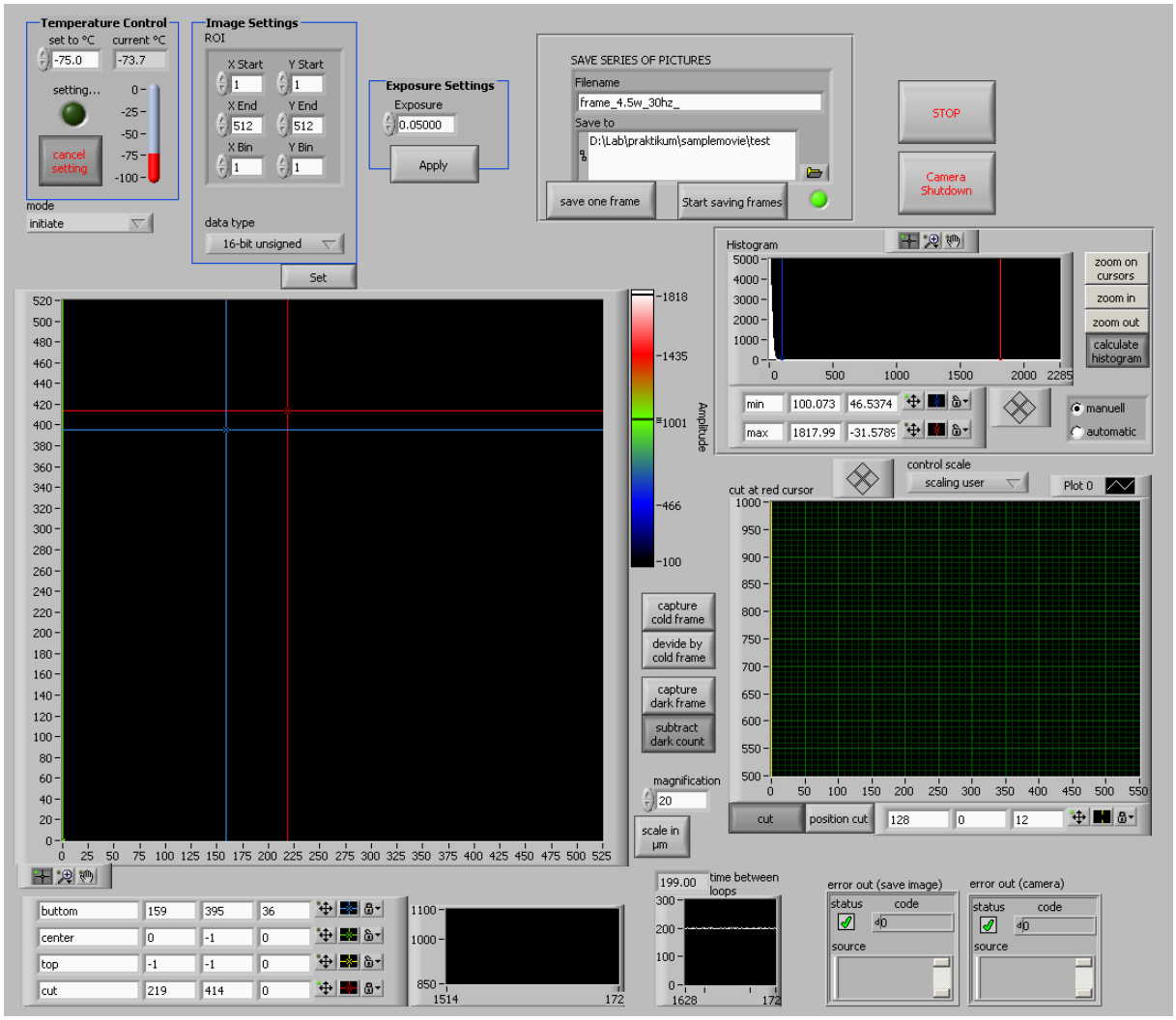
For bead trapping we use a LabView program. The series of images from a measurement are read in one by one and the center position of spots above a threshold in brightness are determined. This list of positions is then brought into correspondence with the position of the beads in the previous frame.

That way, in the end we have a list of the x and y position in every frame for each of the beads. Should the routine loose track of a bead, the trace ends there.

V. Computer Interfaces

We will use two computers of the experiment. The first controls the camera and saves the movie frames, that we will use to extract the particle speeds. The second houses the analog out card. From this the AOD is controlled.

Our custom camera software looks like this:



VI. Preparation

Please familiarize yourself with this introduction. The way the measurements will be done should not be surprising after that. How can we extract the desired values out of the collected data? Software to implement a possible solution are present at the laboratory. Nevertheless it is highly encouraged to come up with your own sketch of an analysis.

VII. Measurement walkthrough

- Switch on the AOD power supplies (2)
- Switch on the camera (if not runnung) start the programs ScanXY (left monitor) and the CascadeII (camera, on the right monitor)
- Switch on the LED driver; set current to about 20mA. You should be able to see the green light under the hood from behind the microscope and on the sample stage
- Start the scan program on the left monitor; don't start the scan yet.
- Pipette 10µl water with fluorescent beads on a microscopy slide. Put the sample under the microscope.
- Focus the sample
- Line up the optical axis of the laser and the camera. For this we use the wide area of effect of convection in a drop of water with fluorescent beads. Turn the key on the laser panel and set the power output to 250mW. The press the emission button. The red light indicates, that the laser is on.
- Make a BCECF chamber. Use 1µl of the Eppendorf tube “F6” (50µM BCECF in 10mM TRIS). Cover this with a 12mm diameter cover slip and seal the chamber with paraffin oil on the sides. Put the sample under the microscope

- Focus the laser with the laser stage z micrometer screw. Take a picture of the spot (static spot hot). Then turn off the laser, wait a moment, and take another picture (static spot cold).
- Turn the laser back on and determine the approximate temperature of the laser spot.
- Start the scan. If you can not see anything, try slowly increasing the laser power output. You should see a ring of illumination
- Next we want to use stroboscopic illumination. Activate the feature in the scan program, set the 'start' and 'stop' point about 1% the '# points' apart, and then press the “update” button to send the new settings to the buffer of the I/O card. Turn the LED illumination down all the way. Next, set the exposure time of the camera to a value much higher than the pattern repeat time, for example 1s. You should see the spot and the path also with lightly diminished fluorescence (why is that?).
- Position the red and blue cross hair in the center of the spot and ahead of it where the temperature is lowest on circle. The pixel values on the position of the cursors are displayed below the live camera preview. Use these values to determine the difference between the peak spot temperature and the temperature, the loop cools down to between passes of the laser, called temperature delta from now on.
- Notice how changing the laser power output and the pattern frequency changes the temperature delta.
- For a more thorough temperature analysis later on, record two sets of pictures for every setting: one with the laser on, one with the laser off. The division by cold frame done by the camera control software is not saved in the recorded frames.
- For our first set of fluid speed measurements we want at least 5 different temperatures between a temperature delta of 2 K to 14 K at a constant pattern frequency of 100Hz. Determine the necessary laser output to create the temperatures and record the values.
- The second set of measurements should be done at constant temperature but different pattern frequencies. Use a temperature delta of about 8K and pick at least 4 frequencies between 20Hz and 200Hz. Record the necessary laser output settings.
- Next make a chamber with the bead liquid. Stick exactly to the procedure you used to create the BCECF chamber to get the same chamber height.

- Reduce the exposure time to 50ms. Put the frame time setting to 200ms. These 200ms will be the time between two frames. Be sure to stick with this or record changes thoroughly, since the velocity calculation depends on it.
- When you scan the laser though the bead fluid, you should make out the liquid motion. Record the motion for all the temperatures and frequencies. In general I recommend to put all parameters of interest into the file name.
- While the movies are recorded, you can start tracking the beads in the finished movies.

Analysis

At the lab the combination unheated and heated snapshot images have to be used to determine the peak spot temperature for the different pump experiments.

The frame stacks of the bead motion needs to be tracked this the particle tracker program available at the lab. The output of this is a tab separated value .txt table for each of the tracked particles.

How the particle speed can be calculated should be discussed and if possible implemented by you. If we run out of time, we can use the prepared program at the laboratory.

Homework

Interpret the different peak velocities and plot the speed against peak temperature or pattern frequency.

Fit the peak velocities and interpret the coefficients.

VIII. References

I.1. E. Yariv and H. Brenner, *Flow animation by unsteady temperature fields*, **Phys. Fluids** 16, L95 (2004).

I.2. F. M. Weinert, J. A. Kraus, T. Franosch, and D. Braun, *Microscale Fluid Flow Induced by Thermoviscous Expansion Along a Traveling Wave*, **Phys. Rev. Lett.** 100, 164501 (2008).

I.3. F. M. Weinert and D. Braun, *Optically driven fluid flow along arbitrary microscale patterns using thermoviscous expansion*, **J. Appl. Phys.** 104, 104701 (2008).