Laser Fibers to Detect and Collect Fluorescent Beads

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Abstract

An optical fiber with a capillary has been used to detect green fluorescent beads using laser induced fluorescence and a vacuum pump has been used to collect the beads into a capillary. As a proof of principle, beads were detected and collected. This was done to eventually be able to apply the same principle to analyze and collect pancreatic cancer cells in real-time, in-vivo.

Furthermore, several optical fibers with different lengths and capillary diameter have been tested to see which one is best suited for further studies with in-vivo. The results indicates that decrease in fiber length and decrease in capillary diameter both decreases the difference in pressure between the two ends of the fiber.

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Contents

1 Introduction

There are more than 331000 deaths per year caused by pancreatic cancer, about 6% of people will live five years after being diagnosed with it [1]. Treatment for cancer in the pancreas is difficult since the pancreas is hard to reach. The pancreas is located in the upper abdomen and is surrounded by organs [2]. With today's methods, pancreatic cancer treatment is time consuming, this means that treatment often happens when it is too to save the patient. Moreover, treatment methods include surgery, chemotherapy and radiotherapy, which are invasive for the body [3]. A new method for pancreatic cancer treatment is in development. This method include optical fibers and is hoped to be able to analyze and collect pancreatic cancer cells in real-time, in-vivo. Since fibers have a small diameter they open the possibility for minimal disturbing measurements for the body in-vivo [4].

1.1 Currently Used Methods for Pancreatic Cancer Treatment

When having surgery to remove pancreatic cancer most of the times only parts of the cancer is removed. Sometimes also organs around the pancreas needs to be removed.

Chemotherapy can be used to stop the cancer from coming back after surgery or before surgery to help make the cancer smaller.

Radiotherapy can be used to treat early pancreatic cancer if there is something that prevents from being able to do surgery. [5]

1.2 Currently Used Methods for Cancer Diagnostics

One method for analyzing cancer cells is called flow cytometry. Flow cytometry can be used to analyze multiple cell parameters. However, this method is both expensive and requires special training in order to use. Flow cytometers consists of a fluidic system and a laser. The cells flow one by one through a detection region. In the detection region they are shone upon by a laser, some of the cells will then fluorescence in a certain manner

and can be characterized from which wave length they fluorescence with. [6]

1.3 Optical Fibers

A laser is a beam of light that is coherent, monochromatic, and collimated. Coherent means in phase, monochromatic means only one wavelength and collimated means that the rays are parallel pointed.

Optical fibers guides a laser in its core, as seen in figure 1. The core has a high refractive index and is covered in a cladding which is an isolating layer. The cladding is covered in a layer called coating, the coating protects the fiber when it is under stress. Inside of the fiber, most of rays remains in the core due to total internal reflection [7]. Total internal reflection is the consequence of a difference in refractive index between the core and the cladding. Nevertheless, some of the light will go to the cladding. The light which have ended up in the cladding will eventually leak out into the coating.

When applied in-vivo the laser could be guided through the fiber despite curvature in the fiber, in contrast to free lasers which can only go in a straight path.

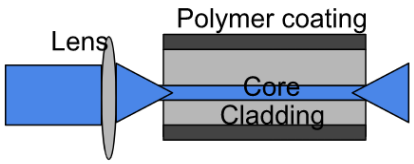


Figure 1: A laser beam is focused into the fiber core through an optical lens. The light propagates along the core and leaves the other fiber end as a divergent beam.

One type of optical fiber is based on silica, with a core of fused silica, a cladding of silica and a coating of polymer. The reason being that silica has a high refractive angle, is not affected by high temperatures, is inert and holds its shape under high pressure. Furthermore, they are cheap to produce relative to instruments that are currently used to treat the types of cancer that this method is aiming to complement.

Some fibers have one or more capillaries, the capillary is a hole in the cladding that goes parallel with the core. It can be used to collect particles.

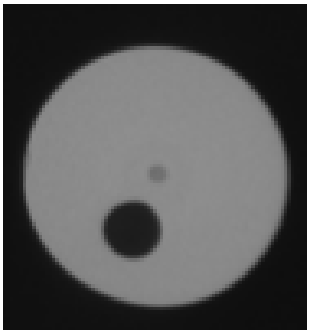


Figure 2: A cross-section of fiber with a core in the center and a capillary underneath.

1.4 Optical Fiber with a Capillary

The principle is that a laser can fluoresce particles in a sample by exciting them. The laser is led in the core of a fiber, when a particle is close enough to the tip of the fiber and the fluorescence signal exceeds a threshold, the particle will activate a vacuum pump. The pump sucks the particle in to a capillary where it is being stored and analyzed. Studies so far have mainly focused on studying fluorescent particles that are not stuck to any tissue, but it is hoped that optical fibers will be applicable for analyzing pancreatic cancer cells that are being stuck on tissue in-vivo [8] by bringing the fiber down to the pancreas through the throat.

When doing experiments with capillaries the fluid flow will depend on the changes in pressure, fluidic resistance and surface area to volume ratio, SAV. SAV is the ratio between the area of the surface that the fluid is inside of and the volume is the volume of fluid. Models used for studying microflow can not be assumed to function the same a devices made for macroflow studies, where gravity will have a more significant impact in the behavior of the fluid [4, 6].

The flow rate of an incompressible liquid, that only changes its viscosity with changes in temperature or pressure can be described by the Hagen–Poiseuille equation (1) if it flows with laminar flow and constant velocity through a straight capillary with constant circular cross-section that is considerably longer than its length. The flow near the entrance of the capillary may be more chaotic than in further in to the capillary, Hagen–Poiseuille equation may not hold close to near to the entrance of the capillary for

that reason.[9]

1.5 Physiological Limitations

A fiber needs to be of a certain length for it to reach the pancreas. When regulating the length of the fiber other variables are affected. There is need for documentation regarding how to control the various variables through in-vitro experiments before the experiments in-vivo can start. A fiber should not be shorter than 0.5 m - 1 m in order to reach the pancreas. Before putting the fiber in the body it will be put another layer of coating. The fiber covered in coating can have a diameter of at most 250 µm in order for it not risking damaging internal organs. Silica based fibers are non-toxic for the body [10] and are therefore the ones that will be used in this experiment.

Cancer cells can withstand a suction pressure of at most 580 Pa and normal pancreatic cells can withstand a suction pressure of at most 1.06 kPa [11]. In order to regulate for that, the pressure at the pump, the length of the fiber and the diameter of the capillary can be adjusted according to Hagen–Poiseuille equation (1). The validity of Hagen–Poiseuille equation applied to optical fibers with capillaries should be experimentally verified.

The minimal capillary diameter is estimated to be 45 µm. The diameter of a cancer cell is between 20 µm and 25 µm [11] and cells will possibly come in accumulations [12], therefore chances are that cancer cells will stick to the capillary walls clogging the capillary with a capillary diameter smaller than 45 µm. Although, in optimal cases, cancer cells can be collected one by one. Whether 45 µm is also too small is to be tested. However, a larger capillary diameter could lead to the upper bond for the diameter being exceeded.

1.6 Aim of Study

The aim of this study is to test how to control suction pressure by the fiber-tip by changing the capillary diameter and the fiber length and to test how the fluorescent signal varies with respect to the distance between the fiber-tip and the fluorescent beads when picking

up beads from a sample using an optic fiber with a capillary. The purpose of this study is to be a proof of principle, to eventually be able to analyze and collect cancer cells using an optic fiber with a capillary.

2 Method

The sample was made by adding Polyethylene glycol-electrolyte, PEG 200 (w/w), a fluid with high viscosity compared to that of water, to a mixture of water and beads with a 1:100 proportion between PEG and water. The proportion between water and PEG was set by testing when the viscosity of the fluid was high enough for the beads not to sink to the bottom of the sample. The sample was placed under a microscope, as seen in figure 3.

2.1 Used Fluorescent Particles

The beads that were used had a diameter of 10 µm and fluoresced with green light when shone upon with a laser. Some beads are fluorescent without addition of other substances. That is not the case for cancer cells. One way of making the cancer cells fluorescent is to tag cancer cells with antibodies with a fluorescent protein, such as green fluorescent protein, GFP. GFP absorbs blue light at 395 nm and 475 nm while emitting green light at 508 nm [13].

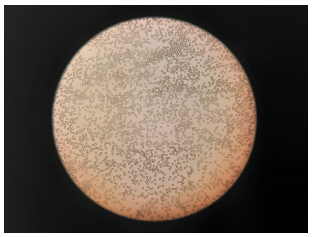


Figure 3: A microscopic picture of a sample of beads.

The coating of the fiber was made removable by adding the fiber to acetone and removed using a scalpel, since light that ends up in the coating is slightly fluorescing

which adds background noise to the measurement. The fiber had a core of fused silica with a diameter of 8 µm, a capillary with a diameter of 30 µm, a cladding of silica and a total diameter of 125 µm.

2.2 Finding the Pressure by the Fiber-Tip

The setup seen in figure 4 was used to measure the flow rate, Q, of a fluid. The capillary was filled with water dyed with fluorescein in order to reduce the capillary forces that would be caused by start measuring Q when the capillary was filled with air. A hydrophobic, transparent immersion oil was sucked into the capillary and Q was measured by observing how far into the capillary the immersion oil went in a specific amount of time. The dynamic viscosity, μ, of the immersion oil was estimated to be $0.014\,\mathrm{Pas}^{-1}$ based on its kinematic viscosity, while considering the density of the oil to be the maximum value that was indicated. From that, the difference in pressure between the two ends of the fiber was calculated using the following equation:

$$\Delta p = \frac{8\mu L Q}{\pi R^4}. \tag{1}$$

Where $\Delta p = p_1 - p_2$,

p_1 = pressure at the end connected to the pump,

p_2 = pressure at the end that was in contact with the sample,

r_c = radius of the capillary,

and L = length of the fiber.

When substituting

$$Q = \frac{\pi r_c^2 l}{t} \tag{2}$$

into equation (1) and solving for l the following expression

$$l = \frac{\Delta p r_c^2}{8\mu L} t \tag{3}$$

was obtained.

Where t = time that the immersion oil was sucked in to the capillary

and l = length that into the capillary that the liquid was sucked in during the time t.

Different fiber lengths and capillary diameters were tested.

2.3 Detecting and Collecting Beads

The experimental setup can be seen in figure 4. A laser sent light with a wave length of 491 nm through the fiber. The excitation beam was launched into the fiber core through a dichroic beam-splitter, that reflected blue light and transmitted green light, and a focusing lens and propagated through the fiber to its tip. The signal from excited beads was collected and guided backwards through the fiber, transmitted through the beam-splitter and filtered and detected by a detector. Backward-scattered laser radiation and other stray light were filtered and color filters placed before the detector, to ensure that only the fluorescence from the beads was detected. A switch device was connected to the vacuum pump used and the fiber. When a fluorescent signal closer than in between 4.25 µm and 9 µm was detected, the threshold exceeded. The switch was controlled with a python program to automatically open when the fluorescence signal exceeded the threshold. Sample was sucked in to the capillary while the switch was open. The switch turned off again after 2 s. The sampling time was set to be 0.2 s after consideration of the accuracy of the measurements and the amount of data stored. The threshold was set to be 0.04 V based on testing from what distance the bead in proximity of the fiber-tip was the bead that was detected.

2.3.1 Estimation of Threshold

The distance that beads which were along the fiber axis were from the fiber-tip was measured. This was done by finding the proportion between the microscope image of the fiber diameter and the real diameter of the fiber and measuring the microscope image of the distance between the fiber-tip and the bead. The distance between the bead in

proximity of the fiber-tip and the fiber-tip was correlated to the signal that was detected. The distance between beads which generated a signal slightly under the threshold and the distance between beads which generated a signal slightly over the threshold was measured. From that an approximated distance that the bead had to be in order to activate the pump was made.

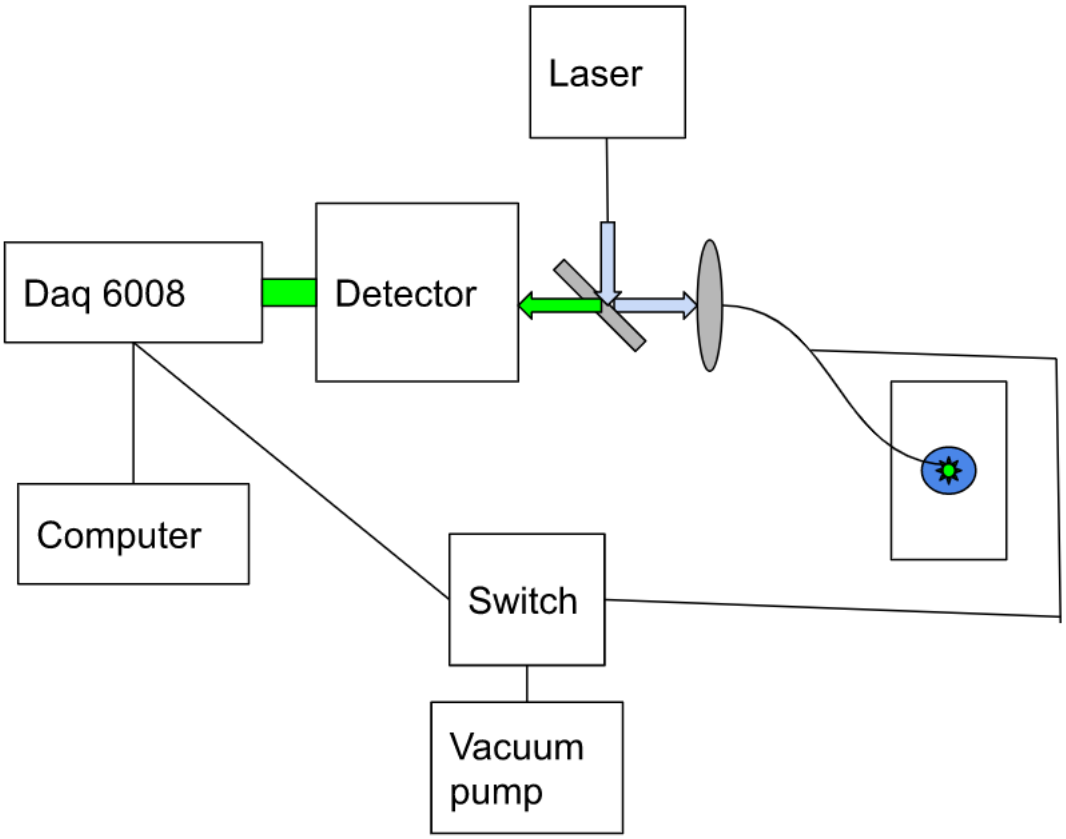


Figure 4: A schematic illustration of the experimental setup.

3 Results

The results are divided into results from the the bead collection experiment and results from the experiment that measured Q through capillaries of different sizes.

3.1 Pressure at Fiber-Tip

By measuring how far into the capillary the hydrophobic liquid goes in different amount of time with given L and r_c, l is found to be dependent on t according to the following linear regressions:

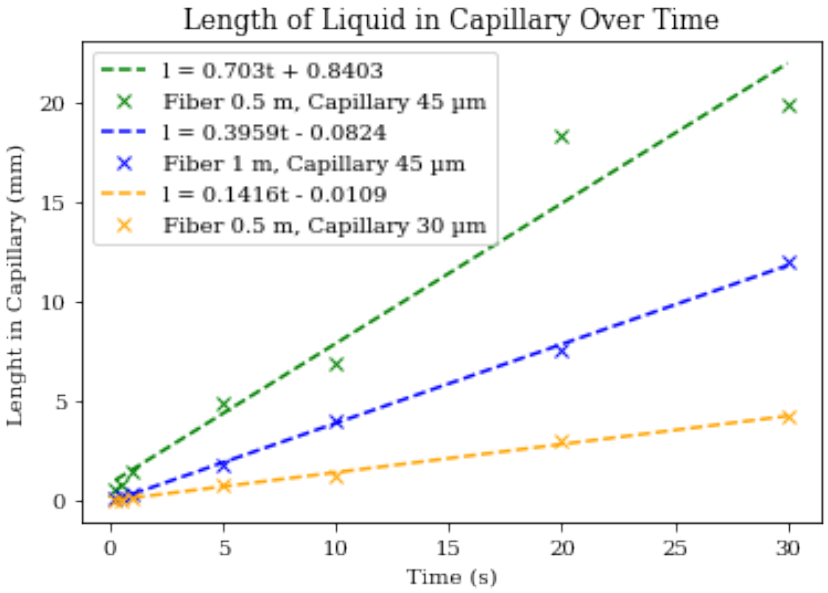


Figure 5: The graph describes how long in to the capillary the immersion oil travels (y-axis) over time (x-axis), when starting from the fiber-tip. Green - fiber with a length of 0.5 m and a capillary diameter of 45 µm. Blue - fiber with a length of 1 m and a capillary diameter of 45 µm. Yellow - fiber with a length of 0.5 m and a capillary diameter of 30 µm.

The functions of the linear regressions from figure 5 corresponds to equation (3). When matching the coefficient in front of t from the green function in figure 5 with the coefficient in front of t from equation (3) this expression is obtained:

$$0.1416 = \frac{\Delta p r_c^2}{8\mu L}. \tag{4}$$

When solving for Δp the change in pressure is found to be 35 kPa. The value of p_1 was set to be 100 kPa. Consequently the pressure in the fiber tip connected to the sample is 65 kPa.

The result from doing the same for the two other linear regressions from figure 5 is

summarized in table 1.

Table 1: The pressure drop for each of the fibers and their correspondent pressure by the fiber-tip.

$L\ [m]$	$d\ [\mu m]$	$p_2\ [kPa]$	$\Delta p\ [kPa]$
0.5	45	22	78
0.5	30	65	35
1.0	45	12	88

Where $d = 2r_c$.

There was a decrease in Δp for smaller values of L and a decrease in Δp for smaller values of d.

3.2 Detected and Collected Beads

Beads were collected from the sample as seen in figure 7. The detected fluorescent signal was shown in real time and it is shown in figure 6. The red horizontal line in figure 6 is the threshold.

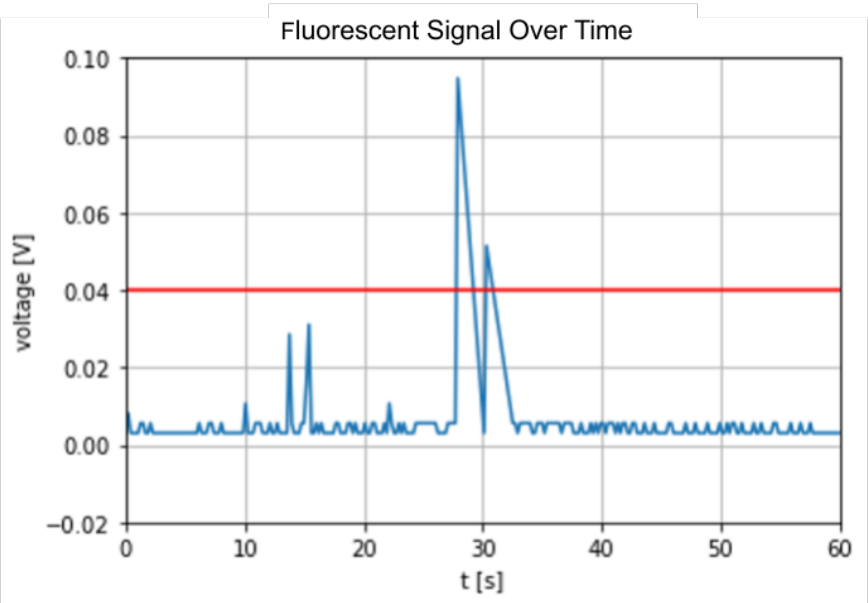
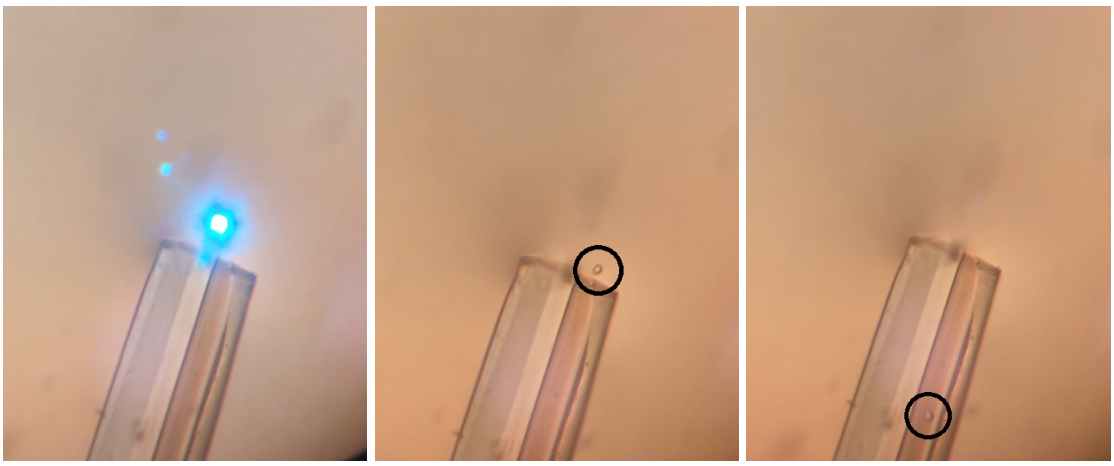


Figure 6: The light signal is converted to an electrical voltage. The graph shows the signal that is being detected over time.

The peak in figure 6 at 28 s corresponds to the bead that is being collected in figure 7.



(a) A bead is detected. (b) The pump activates. (c) The bead is collected.

Figure 7: A bead is detected and collected.

The distance that the bead detected after 16 s in figure 6 was from the fiber-tip was measured to be 9 µm. The distance that the bead detected after 28 s in figure 6 was measured to be 4 µm. Both the bead detected after 16 s and the bead detected after 28 s was along the fiber axis.

4 Discussion

The optical fiber with a capillary was able to suck in beads as seen in figure 7. However there were difficulties when trying to get a bead close enough to the fiber-tip for it to activate the pump.

4.1 Physiological Aspects

When eventually applying this principle in-vivo the viscosity of the fluid will be more similar to that of water at 37 °C, 1 mPas^{-}1, which means that the difference in pressure between the ends of the fiber will be lower.

To be able to efficiently make predictions for p_2 without need for calculations of Δp,

Δp should be neglect in comparison with p_1. That is to say a capillary that minimizes Δp is optimal. According to table 1, Δp is minimized with a value of L that is close to the lower physiological bound and d close to the lower physiological bound. According to equation (1) on the other hand L should be close to the lower physiological bound and d should be close to the upper bound. When transitioning to cancer cells instead of beads it is of importance to know what pressure the cell will experience while being sucked up in order not to damage it. Therefore, this should be studied until there are no ambiguities regarding what bound d should be close to in order to minimize Δp.

4.2 Restrictions to the Setup

Firstly, the beads tended to sink towards the bottom of the sample, where they were hard to reach with the fiber-tip. That could be solved by having a shaker to keep particles in movement. Secondly, the fiber-tip was moved manually, which meant that the precision that could be archived was limited. One way of making it easier to get close to the bead could be to control the fiber-tip with a joystick.

The threshold for activating the pump was set by considering the background noise and the concentration of the beads. The background noice was estimated to be less than 0.01 V. A threshold of 0.04 V was found to be working for the sample that was used. The maximum distance that a bead along the fiber axis had to be in order to be collected was in between 4 µm and 9 µm. From that the maximum concentration of beads was restricted, considering that a certain amount of liquid will come with the bead when the bead is collected and no other bead could be in that volume in order to pick up exactly one bead for each detection.

The fiber that was used had a diameter of 30 µm. That could be increased to 45 µm when applying this method to cells instead of beads. Since cancer cells typically have a diameter of 20 µm a capillary diameter of 45 µm would prevent clogging the capillary if the cancer detected cancer cell stick to another cell when being sucked up.

4.2.1 Measurement of Pressure by the Fiber-Tip

The difference in pressure increased when d increased for constant L while it was expected to decrease according to equation (4). By fixing Δp for $L = 0.5\,\text{m}$ and $d = 30\,\mu\text{m}$ or Δp for $L = 0.5\,\text{m}$ and $d = 45\,\mu\text{m}$ and solving for the other the the difference between the theoretical value and the measured value varies with 72 kPa and 140 kPa respectively. The reason for this could be that the measurements were under conditions that not matched with Hagen-Poiseuille equation (1). The equation holds only for situations where the flow has stabilized. The measured flow was by the entrance of the fiber and and happened soon after the pump was activated, which means that it may not was the case that the flow had stabilized during the measurement. However, the small area of the cross-section of the capillary was expected to stabilize the flow during the measurement.

Another explanation could be that the accuracy of the measurements was too low and that the true values of l gives values for Δp that matches Hagen-Poiseuille equation (1). One potential source of error was that the capillary forces may affected the flow after the pump was turned off. Another source of error could be that the length of either the fiber with $d = 30\,\mu\text{m}$ or the fiber with $d = 45\,\mu\text{m}$ was had a smaller value of l than expected. One reason to think that the fiber with $d = 45\,\mu\text{m}$ and an expected value of $L = 0.5\,\text{m}$ not had a value of $L = 0.5\,\text{m}$ is that the systematic source of error seems to be about ten times higher for for the measurements of the flow rate for that fiber than for the measurements of the flow rate for the other two fibers, as seen in figure 5.

4.3 Further Research

Further research needs to be done to test this method in in-vitro environments that are more similar to that of the pancreas. The conditions with cells are different from those with beads. The beads that were used were are eight times smaller than an average cancer cell. Further studies may use tagged cells instead of beads.

4.4 Conclusion

It has been shown that optical fibers with a capillary can be used for detecting and collecting fluorescent particles. Further, it has been shown that Δp is smaller for a shorter fiber length. However, the increase in capillary radius did not get expected results and needs to be measured again before conclusions can be drawn regarding what capillary size to go further with.

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