Detecting and Collecting Pancreatic Cancer Cells Using Fiber Optics

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Abstract

Currently, the detection and diagnosis of pancreatic cancer requires extensive procedures. Most involves collecting biopsies for determination of the health of the cell. By implementing techniques using optic fibers together with laser, detection of cancer cells could be profoundly simplified. This study investigated the method where laser is shone on cells and the reflective light is detected. If the reflected light indicates a cancer cell, hypothetically via a green fluorescent protein or polarized light scattering spectroscopy, a pump would turn on and suck it into a capillary in the fiber for collection. The aim of this study was to test if this method works in practice and to investigate how pressure drop through a capillary is connected to changeable variables of the fiber. Green fluorescent beads were detected and sucked into a capillary using blue laser, conveying that the method was in practice possible to use for detection and collection of particles. Further studies should include how applicable the Hagen-Poiseuille equation is when using microfluidics and fiber optics. The method described here could possibly be automated. Further work is needed for finding the optimal values for use of fiber optics technique in healthcare.

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Contents

1 Introduction

Cancer is often detected when it is already too late to cure [1]. Symptoms are often vague [2], and current detection methods rely on the patient contacting the healthcare system when falling ill, resulting in a diagnosis when in many cases time for effective treatment has already passed [3]. This is particularly true for pancreatic cancer [4]. It eludes early detection because it is difficult to reach, being located in the upper abdomen and surrounded by other organs [5]. A novel approach utilising fiber optics in cancer detection may simplify this process.

1.1 Optic Fiber

Optic fibers are thin, cylindrical tubes mainly used to guide light. They are commonly made of silica glass and consist of a core, a cladding, and a jacket, see Figure 1 [6]. The core has a greater refractive index than the cladding, making light travel slower in the core and faster in the cladding, which causes a change in direction when light travels between them [7]. The difference in refractive index is crucial for guiding light because it generates a total internal reflection when light meets the core cladding boundary at a sufficient angle [8]. This is used when guiding and focusing monochromatic light in lasers [7]. Silica glass is durable and flexible, non-toxic for the body and water resistant. Further, it has sufficient sensitivity, acceptable signal fading and can be used for procedures *in vivo*, making it suitable for cancer detection [9].

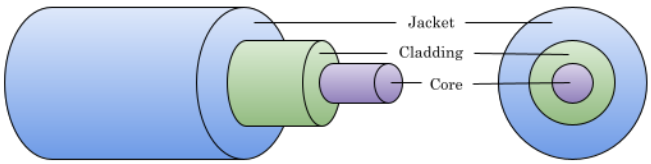


Figure 1: The structure of a fiber.

So called specialty fibers can have longitudinal holes integrated in the fiber called capillaries, giving it different abilities beyond guiding light [10]. In a capillary, particles and liquids can be stored, and with pressure sucked or pushed through, making it possible to collect small particles like cells [6].

1.2 Microfluidics

It is of importance to study microfluidics, the dynamic of very small amounts of fluids in a microenvironment, to accurately predict how liquids and capillaries act and work together [11]. This is because fluids in microchannels act differently than in larger channels, since different forces dominate on smaller length scales [12]. The mass of fluid inside a fiber is relatively small, allowing capillary forces and surface tension to dominate while gravity is comparatively insignificant [13]. The frictional forces in the capillary cause a pressure drop between ends of fiber when carrying fluid [14].

1.3 Optic Fiber in Healthcare

Optic fibers could be used as an effective way to detect pancreatic cancer through laser-induced fluorescence [15]. There are different ways to analyse cells based on emitted light. One way is to induce cell fluorescence when cells are shone upon with a specific light [16]. This is done, for instance, by tagging cancer cells with antibodies containing a fluorescent protein [17]. Tagging can be done with green fluorescent protein, which absorbs blue light at wavelengths of 395 nm and 475 nm, while emitting green light at 508 nm [18]. Other methods, like PLSS, analyse the morphology of cells in terms of their scattered light [19].

It is of importance to take into account the length and the diameter of the fiber used in procedures *in vivo* to make it as noninvasive in the body as possible, while still being able to collect pancreatic cells. The diameter of a pancreatic cell is around 16 μm [4] and it can withstand a pressure of just over 2 kPa [20]. Cancer cells are usually more adhesive than

regular cells and will therefore possibly come in accumulations [21]. This increases the risk of clogging if the capillary is too small. The minimal estimated capillary diameter in the fiber is therefore 30 μm. The length of fiber needed to reach the pancreas is estimated to be 0.5 m.

1.4 Aim of Study

The general purpose of this study was to determine if fiber optics can be used to detect and collect fluorescent beads *in vitro*. This to make a proof of principle if this method can be applied in future procedures *in vivo* using cancer cells. The aim of the primary experiment was to investigate how the pressure drop over a fiber was dependent on length of the fiber and diameter of the capillary. This to also see how the Hagen-Poiseuille equation corresponded to reality.

2 Method

Two different experiments were carried out. The aim of the primary experiment was to find the optimal fiber dimensions able to create the pressure drop cancer cells can withstand. The final experiment tested if it was possible to detect and collect a 10 µm bead using fiber optics and laser.

2.1 Pressure at the Fiber Tip

Predicted pressure drop over the ends of a capillary in a fiber were calculated using the Hagen-Poiseuille equation,

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

Here, $\Delta p = p_1 - p_2$, where p_1 is the pressure at the end connected to the pump and p_2 is the pressure at the end in the sample. μ is the viscosity of the liquid being sucked, Q is

the flow rate, R is the radius of the capillary used, and L is the total length of the fiber used. When substituting

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

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

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

was obtained.

When having an experiment testing different l over t, the slope, k, of the linear function $l(t)$ can be written as

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

In equation (1) can be seen that higher flow velocity, higher viscosity, and greater length lead to a greater pressure drop between two ends of the fiber, while a larger radius decreases the difference. The accuracy of the equation remains to be experimentally verified when using microfluidics. In this study, the fiber lenghts 0.5 and 1 m were used together with capillaries with diameters of 30 μm and 45 μm.

One end of the fiber was connected to a pump, and the other end was put in Flourescein colored water to be able to see the liquid being sucked. The pump was turned on and the capillary was filled with the colored liquid to reduce the capillary forces when obtaining Q. The pump was turned off, and the end in the colored liquid was put in a translucent hydrophobic liquid with close to the same viscosity as the dyed water and the pump was on for 30 seconds. The length of which the translucent liquid filled the capillary was measured, the end was put in dyed water and the pump was on for 20 seconds. The same

was done three times for each of the intervals $30, 20, 10, 5$, and 1 seconds. When measuring the length the liquid travelled for 0.25 seconds, the pump was put on for 0.25 seconds and turned off automatically, and turned on manually after 1 second for 0.25 seconds. This was done 10 times in a row for 0.25 seconds and 5 times in a row for 0.5 seconds. The total length the liquid had travelled was measured and divided by 10 respectively 5. This was repeated twice for each of the time intervals.

2.2 Detecting and Collecting Beads

When suitable values of length and diameter of capillary was found, the final experiment was performed using a fiber length of 0.5 m with a total diameter of 125 μm, a 8 μm core diameter, and a 30 μm capillary diameter, see Figure 2. A program that controlled the pump manually as well as automatically if a threshold was reached was programmed using Python. One end of the fiber was connected to the pump, and the other end was put in a sample with green fluorescent beads on a glass slide under a microscope. The sample contained 10 μm green fluorescent beads, water and PEG 200 (w/w) with the proportion of 1 : 100 to make the sample more viscous, avoiding the beads to sink quickly to the ground. Blue laser was turned on and guided through the fiber to the sample. When beads were hit at a sufficient distance, they emitted green light that was guided back through the fiber to a detector. If the signal threshold of 0.04 V was reached, the pump was turned on for 2 seconds and the bead was sucked into the capillary. See Figure 3 for a schematic method.

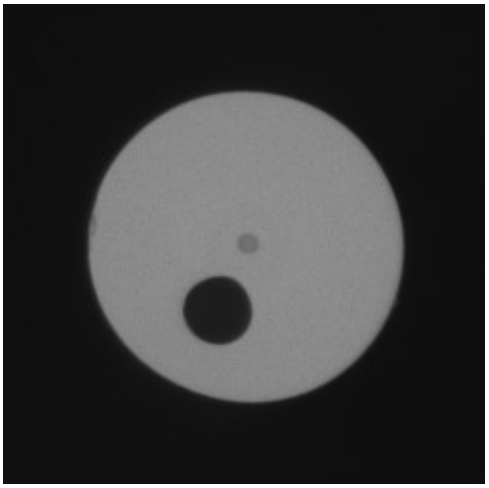


Figure 2: Cross section of the fiber used in the final experiment. It has a 125 μm diameter, 8 μm core diameter, and a 30 μm capillary diameter.

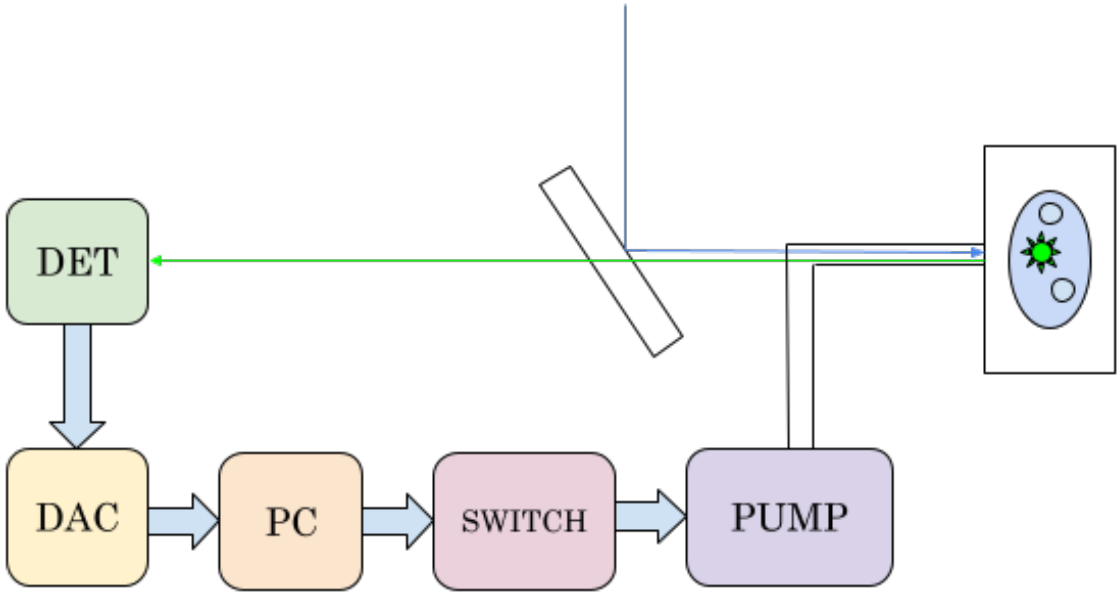


Figure 3: Blue laser is guided through a fiber to a blue light reflective mirror which directs it to the sample. When a bead receives blue light, it emitts green light, which is guided through the fiber, through the mirror, to a detector, DET. The output signal from the detector is guided to a hardware, DAC, which gives the fluorescence signals to the computer, PC. When the threshold is reached, the computer gives a signal to the switch, which starts the pump connected to the capillary. A bead is then sucked into the capillary.

3 Results

The results are divided between the primary experiment in which the pressure drop was tested and calculated using using different variables, and the final experiment, which tested the possibility to suck beads through a 0.5 m and 30 μm capillary.

3.1 Pressure at the Fiber Tip

The results, that can be seen in Figure 4, show the length of travelled liquid into a capillary as a function over the time intervals the pump was on. Liquid travelled longer per time interval in the 0.5 m fiber with a 45 μm diameter capillary than in the 1 m fiber with a 45 μm diameter capillary, and shorter per time interval in the 0.5 m fiber with a 30 μm diameter capillary. The pressure drop and the pressure at the fiber tip for each of the fibers can be seen in Table 1.

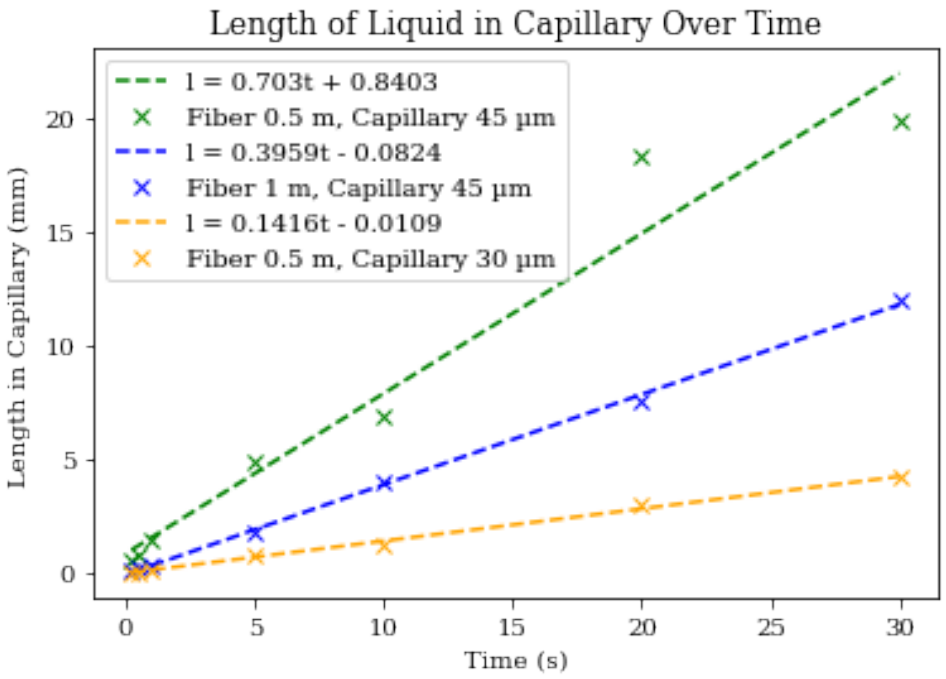


Figure 4: The length of the liquid (y-axis) that has traveled over different time intervals (x-axis), when starting from the fiber-tip. Green: fiber with a length of 0.5 m and a capillary diameter of 45 μm. Blue: fiber with a length of 1 m and a capillary diameter of 45 μm. Yellow: fiber with a length of 0.5 m and a capillary diameter of 30 μm.

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

$L\ (m)$	$d\ (\mu m)$	$\Delta p\ (kPa)$	$P_2\ (kPa)$
1	45	88	12
0.5	45	78	22
0.5	30	35	65

3.2 Detecting and Collecting Beads

Beads were collected from the sample using the 0.5 m fiber with a 30 μm diameter capillary. The peak at 28 seconds in Figure 5 corresponds to a bead being detected and collected in the sequence Figures 6a 6b, and 6c. The bead reaches a signal of 0.95 V when being 4 μm away from the fiber tip.

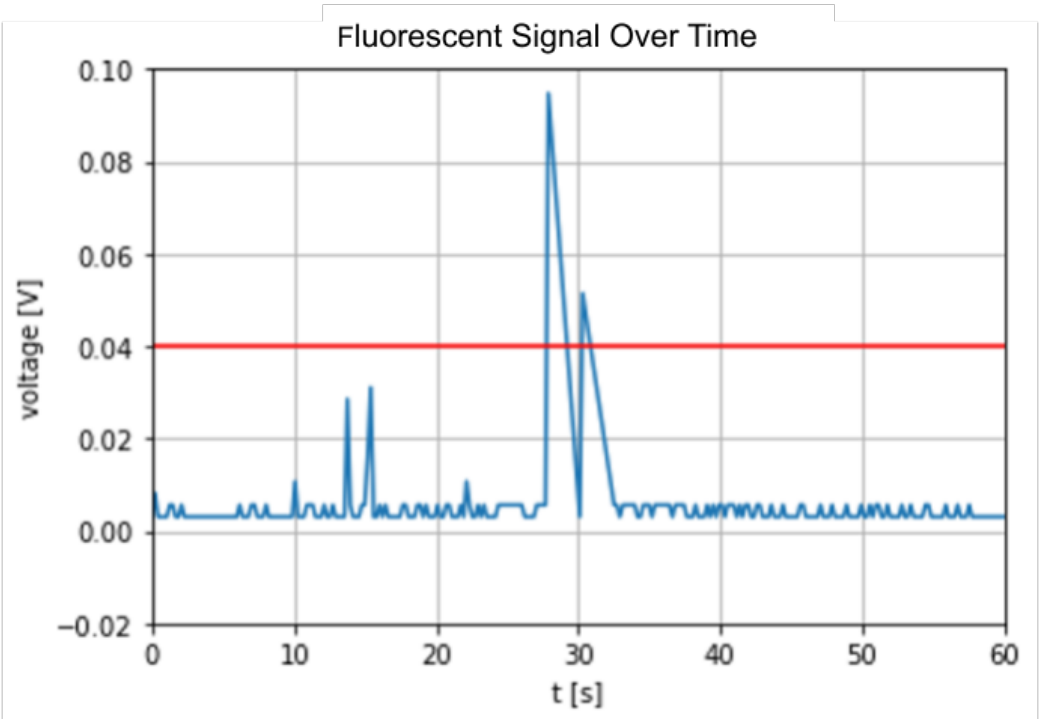
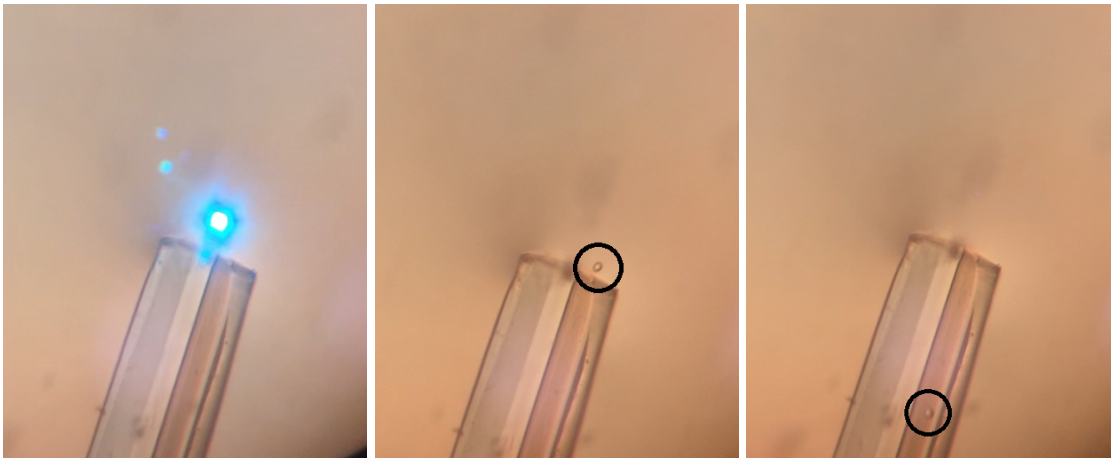


Figure 5: Detection values during 60 seconds in which a bead is detected with a signal over the threshold and sucked through the pump.



(a) Shining bead (b) Bead sucked (c) Bead sucked further

Figure 6: A bead being detected and sucked into the fiber. The bead is fluorescing in Figure 6a and can be seen in the black circle in Figure 6b and in Figure 6c

4 Discussion

The discussion is, as in previous sections, divided between the primary experiment, in which the pressure drop was calculated, and the final experiment that tested the hypothetical method of detecting beads.

4.1 Finding the Pressure at the Fiber Tip

The calculated pressure drop showed that for using a 45 μm diameter capillary, a longer fiber length lead to a higher pressure drop through the fiber, see Table 1. This matched the Hagen-Poiseuille equation (1), which shows that a longer fiber length would lead to a greater pressure drop. However, the values and the pressure drop when using a 30 μm diameter capillary and a 0.5 m fiber did not correspond to the calculations using the same equation. The pressure drop was less than that for the capillary with the same length but with a larger diameter, which was inconsistent with the Hagen-Poiseuille equation (1), which instead shows that a larger capillary diameter should decrease the pressure drop. Sources of error may be that the length of liquid sucked through the capillary always was calculated at the beginning of the fiber. The flow may be more chaotic at the entrance tip than in the rest of the fiber, making the values less accurate than they would have

been further in. Additionally, the time in which the pump was on may have been too short for the pressure to stabilize, leading to inaccurate values, since the equation is used for constant pressure.

When in the body, it is necessary to control the pressure with accuracy to not destroy cells before inspection. The current results show that the 0.5 m fiber and the 30 μm diameter capillary has the least pressure drop, and therefore needing less amount of pressure used to reach the optimal pressure at the sample tip. This was the fiber used in the final experiment.

4.2 Detecting and Collecting Beads

The results from the final experiment show that it is possible to suck 10 μm green fluorescent beads through a 0.5 m fiber using a 0.04 V threshold. However, more studies are needed to apply this to healthcare and detection of cancer cells in the body.

When applying this method to procedures *in vivo*, it has to be noted that this experiment was made while looking into a microscope. It was therefore possible to find beads before they were detected using the blue laser and manually move the fiber to beads. When in the body, this will instead require an automated program with higher precision to reach and detect the wanted cells. The particles needed to be less than $4.25 \cdot 10^{-6}$ m away from the detector to be detected and collected, making preciseness a crucial factor in this study. Since the fiber had to be moved manually, the set-up for this experiment was not optimal from a precision perspective.

The diameter of 30 μm may be too small for *in vivo* procedures. Two pancreatic cells would not be able to be sucked through while sticking together, increasing the risk of clogging. The experiment needs to be done testing more sizes of capillaries, ranging in diameter from 35 μm to 55 μm or higher, while still keeping the total diameter of the fiber

as small as possible.

In the sample, beads sank quickly to the ground of the glass slide due to their size in a not so viscous liquid. This made detection difficult, since there was a short time in which the experiment could be done. When at the ground, the laser shone over the 10 μm beads even when the fiber of 125 μm laid straight to the ground. The core of the fiber that guides the laser is in the centre, and therefore shining over the beads, making it impossible to detect a fluorescent signal at a close enough distance.

4.3 Further research

Further studies are needed to investigate for what variables the Hagen-Poisuelle equation is valid, and to see if it can be applied to micro channels. It is of importance to be able to calculate the drop in pressure accuratly to stay withing the limitations of pressure in cancer cells. The method needs to be tested *in vitro* in environments that are similar to that of the pancreas, and real cells instead of beads have to be used before applying this method to healthcare.

For an automated program depending on the fluorescence of cells, methods for detection and separation between cancer cells and regular cells are needed. The distance between the cell and the fiber that is possible both for detection and for guaranteed collection needs to be studied further, as well as an optimal threshold.

In this experiment, free beads were sucked from the sample. In the body, the cancer cells will most likely stick together in a tumor. This differ from the environment in which the beads were collected. Therefore, a method to separating the cells before being collected needs to be found and tested to develop this method even further.

4.4 Conclusion

This study showed that pressure drop decreases when using a shorter length of fiber. However, the decrease in capillary diameter caused unexpected results. Further research is necessary before drawing conclusions on what capillary size should be gone forward with and how accurate the Hagen-Poiseuille equation is to reality. Additional research may utilize the results from this experiment when developing this method further. Since a bead was detected and collected in vitro using optic fibers and laser, the conclusion can be drawn that this method can be applied in healthcare and used to detect pancreatic cancer in the future.

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