APP N17-3

Engineering 1282H

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Team Y4

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Parke 12:40 PM

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a.What will your device diagnose? Why is this device needed and what impact will it have?

Our device will diagnose the presence of human immunodeficiency virus (HIV) in humans. HIV kills approximately 800,000 people worldwide, so its study is key for promoting human life [9]. In 2018, 8.1 million people worldwide did not know they were living with HIV [9]. Only 79% of all infected people worldwide know their status [9]. Since testing of HIV is key to treating and preventing new infections, our group chose to create a device to increase the ease and availability of testing. Our device will eliminate the need for any complex equipment and will provide easy and affordable testing to communities with low access to health care. With this new technology, more people will have access to testing and can get treatment sooner. [10]

b.What type of analyte will you assess (e.g., cell shape, cell surface proteins, DNA)? Why was this analyte chosen?

We will assess the p24 protein. This analyte was chosen as it is a protein only present in blood infected with HIV. When a sample is introduced to a solution containing gold nanoparticles, hydrogen peroxide, monoclonal anti-p24, polyclonal anti-p24, and streptavidin-catalase conjugate, the solution will turn red if the p24 protein is not present and blue if the p24 protein is present. [5] This happens because when p24 is not present, the hydrogen peroxide reduces the gold in a redox reaction, which leads to the nanoparticles not aggregating, which causes a red solution. When p24 is present, its reaction with the antibodies and the streptavidin-catalase conjugate causes the hydrogen peroxide to decompose, meaning the gold is no longer reduced, and the gold nanoparticles are free to aggregate, causing a blue colored solution. [5]

c.How will your device isolate the analyte? For example, will you isolate the target analyte from the other blood components or analyze the whole blood mixture? Why? How will the device avoid false positives and false negatives?

The device will not entirely isolate the analyte; it will filter the blood in order to obtain plasma. This is because the color change caused by the presence p24 protein would not be very visible in untreated blood. This filtration will be done via bifurcation, as there will be multiple channels with a lower flow rate attached to the main channel that lead to plasma flowing into these lower flow rate channels. This happens due to the law of bifurcation, which states that the large red blood cells will stay in the higher flow rate channel. [9] The device will avoid false positives and negatives simply by the nature of the detection method. The anti p24 in the reagent will not react with any proteins other than the p24 protein, so there will be no false positives, and this method is effective at extremely low concentrations, which will minimize false negatives. [5]

d.How will the blood sample and reagents be loaded into the NANOLYSER?

The blood sample will be loaded into the device using a peristaltic pump attached to a syringe. This pump is constructed of 3 PDMS layers, with 3 valves. An EMF generator sends a negative waveform through the pneumatic layer, which causes the PDMS to relax and allows the fluid to flow through. [3] Then, once this wave has passed, the PDMS constricts, forcing fluid through the valved chambers, meaning it operates similarly to how a heart would pump blood [3]. These chambers are also designed in a way that eliminates backflow [3]. This specific pump was

chosen over other pumps such as piezoelectric pumps or microthrottle pumps as the peristaltic pump is more accurate and controllable, and is completely external, requiring no internal mechanical components of the chip. This kept the chip design simpler and more efficient, and made the peristaltic pump the most viable option. The syringe will insert into the chip inlet, while the peristaltic pump will pump the blood through this syringe and through the channel. This method was chosen, as syringes are expected to already be readily available in any medical setting, are already easily mass produced, and are cost efficient. Therefore, we decided that shaping the fluid inlet to accommodate for a syringe and using it as the insertion method would be most advantageous.

e.How will samples and reagents be moved around and mixed in the NANOLYSER? Describe any methods being considered and the advantages and disadvantages associated with each. Do you have a decision-making strategy for choosing a moving/mixing method?

The blood will be moved through the plasma separation chamber via the use of a peristaltic micropump. The blood sample will first be loaded into the pump, then pumped into the chip through a syringe, and the peristaltic micropump will continue to pump the fluid through the bifurcation chamber of the channel. These peristaltic micropumps have great precision, low cost, and require no extra hardware to install. [3] This precision is important, as varying flow rates may have unexpected and unwanted consequences on flow rate. A low cost is important as we want this chip to be used in developing regions of the world, making price an extremely important factor. The lack of additional hardware makes it simpler, and safer to clean, as there is no need to worry about cleaning solution damaging this hardware. Samples will be mixed by adding texturing to the bottom of the detection chamber [12]. This method adds no complexity to the chip itself, and is the simplest solution to the problem that still provides effective mixing. This method was chosen over other methods like mechanical mixing or acoustic mixing because they add extra technological components that would increase the price, while methods like stream splitting would require additional length be added to the chip and most likely an additional chamber dedicated to mixing, which was undesirable. [10] Therefore, texturing was the solution that fit the best with our goals of being affordable, simple, and easy to operate.

In terms of decision-making, our strategy is typically to evaluate all the options based on their cost effectiveness, simplicity, and lack of mechanical components. We want our chip to be used in developing regions, so cost effectiveness is key. We want our chip to be easy to use and construct as this will make widespread use easier, which makes simplicity important. Finally, a lack of mechanical components greatly cheapens the construction and makes it more reusable as there is a much lower chance of components breaking down over time or being damaged by cleaning. This reusability is what makes the high initial investment required for micromachining feasible. [7]

f.What will be different processing steps (e.g., cell separation, lysis, DNA labeling)? Will these be performed in the same location or in different chambers? Describe any detection methods being considered and the advantages and disadvantages associated with each.

The first processing step will be separation, as red blood cells will be separated from the blood via bifurcation [11]. Using a channel with 5 bifurcation microchannels inside, this separation occurs from the instant the blood enters the chip. Plasma separated from blood will flow into these bifurcation channels and into a separate collection chamber. This method was by far the simplest and most complete of any plasma separation methods available as it could easily be incorporated into the chip and requires no additional processing.

The detection method being used is the observation of a color shift. No other detection methods were been considered as this method was found to be extremely innovative and executable. Compared to other methods, it is simple, fast, and still very effective. [5]

g.How will the NANOLYSER be read (e.g., fluorescence reader)? Consider how the analyte will be detected and how this could be translated into an output that the user can read.

The NANOLYSER will come with a sheet of specific color ranges that indicate whether the test is positive or negative, in order to avoid any subjective errors. These color changes occur due to the redox reaction or lack thereof between the gold nanoparticles and hydrogen peroxide, which is explained more in depth in question b [5]. Since the color will be easy to see, no additional treatments or vision aids will be needed to analyze the chip.

h.Will the NANOLYSER be disposable or reusable?

The NANOLYSER will be reusable; it will have a top and bottom part that can be separated for cleaning and reuse. Although we could have made it disposable, this would make it much less cost effective as production of any lab on a chip with microchannels will be expensive [7], so making it reusable is much better for the consumer. The cleaning will be done by soaking the chip in Tergazyme for no fewer than 20 minutes. Tergazyme is a cleaner typically used to clean hospital instruments, laboratory ware, and a host of other items, and completely removes blood and body fluids from plastic, which is the material of our chip, making it extremely effective [8].

i.What material(s) will your device be made of?

The device will be made almost entirely of PMMA, a hard, clear plastic. Since it is cheap, clear, and easy to work with, it is the ideal material for our chip.

j.How will your device be fabricated?

The device will be made using UV laser micromachining, a production technique that provides quality product and works at scale. Although this method would require a high initial investment, it allows for the mass production of our design, which would be important for widescale use, and also would lead to a decrease in cost per unit. [7]

Blood Processing Algorithm:

1. Collect 1-2 mL of blood using a new syringe, ensuring the environment is sanitary. This is gather a sample that will be tested.
2. Insert sample from syringe into peristaltic pump tubing of peristaltic pump.
3. Pump sample into inlet through secondary syringe that connects pump and chip inlet. Steps 2 and 3 are meant to put the blood sample into the chip, in order for it to be tested.
4. Blood flows through the 5 bifurcation channels in order to filter blood and obtain plasma
5. Due to bifurcation, red blood cells continue to flow down main channel into separate chamber
6. Plasma enters side channels due to bifurcation and flows off to separate chamber
7. Plasma all gathered in one chamber. It is gathered because plasma is the targetted content intended for testing.
8. Plasma pumped into detection chamber manually using a finger pump. This is to move the plasma into the correct chamber for testing.
9. The presence or lack of p24 protein in plasma causes a color shift in detection reagent
10. Color shift is observed by human eye and compared to key
11. Test is determined to be positive or negative based on color change, color change will be easily seen by human eye

Description of How Results will be Read and Diagnosis Made:

The results will be read by comparing the color change in the detection chamber to a provided key. This will be possible as the color change is very pronounced even at extremely low concentrations. This key will be prepared beforehand as a part of the team's research, as it will not be pure red in the absence of p24 and blue in the presence of p24, as plasma is not completely clear. However, these color changes should still be clearly visible, and before distribution the colors corresponding to positive and negative tests will be made into a key with pictures.

Bill of Materials:

Material	Item Number	Supplier	Price
PMMA	**P-051-1ML**	Cerilliant	$39.30 / 1ml
PDMS	**481688-10ML**	Sigma-Alderich	$49.20 / 10 ml
Peristaltic Pump	**Z690171-1EA**	Welch	$1110.00
3 mL Luer-Lock Non-Inert syringe	SYR-NJ3LLBX	New Era Pump Systems	$25.00/ 100 syringes in 1 box
Gold Nanoparticles	**741957-25ML**	Sigma-Alderich	$109.00 / 25ml
Screw and Nuts	43163	Grip on Tools	$7.49 / 240 Pieces

References:

[1]Aldrich: Analytical, Biology, Chemistry & Materials Science products and services. (n.d.). https://www.sigmaaldrich.com/

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[5] de la Rica, R., Stevens, M. "Plasmonic ELISA for the ultrasensitive detection of disease biomarkers with the naked eye." *Nature Nanotech* 7 (2012): 821–824.

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[8] "Tergazyme Enzyme Active Powdered Detergent." *Alconox, Inc.*, Alconox, Inc., 19 Apr. 2017, alconox.com/tergazyme/.

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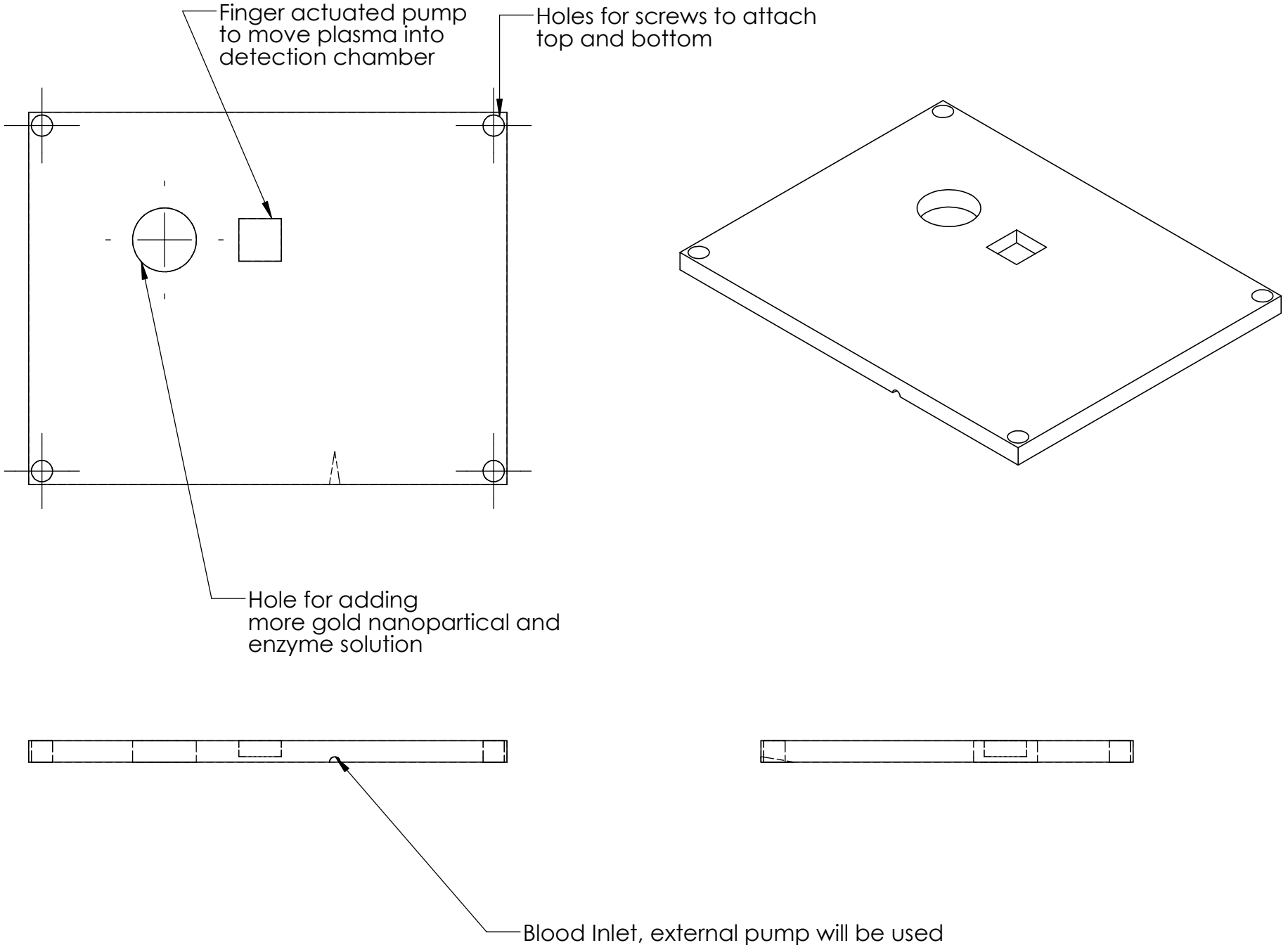
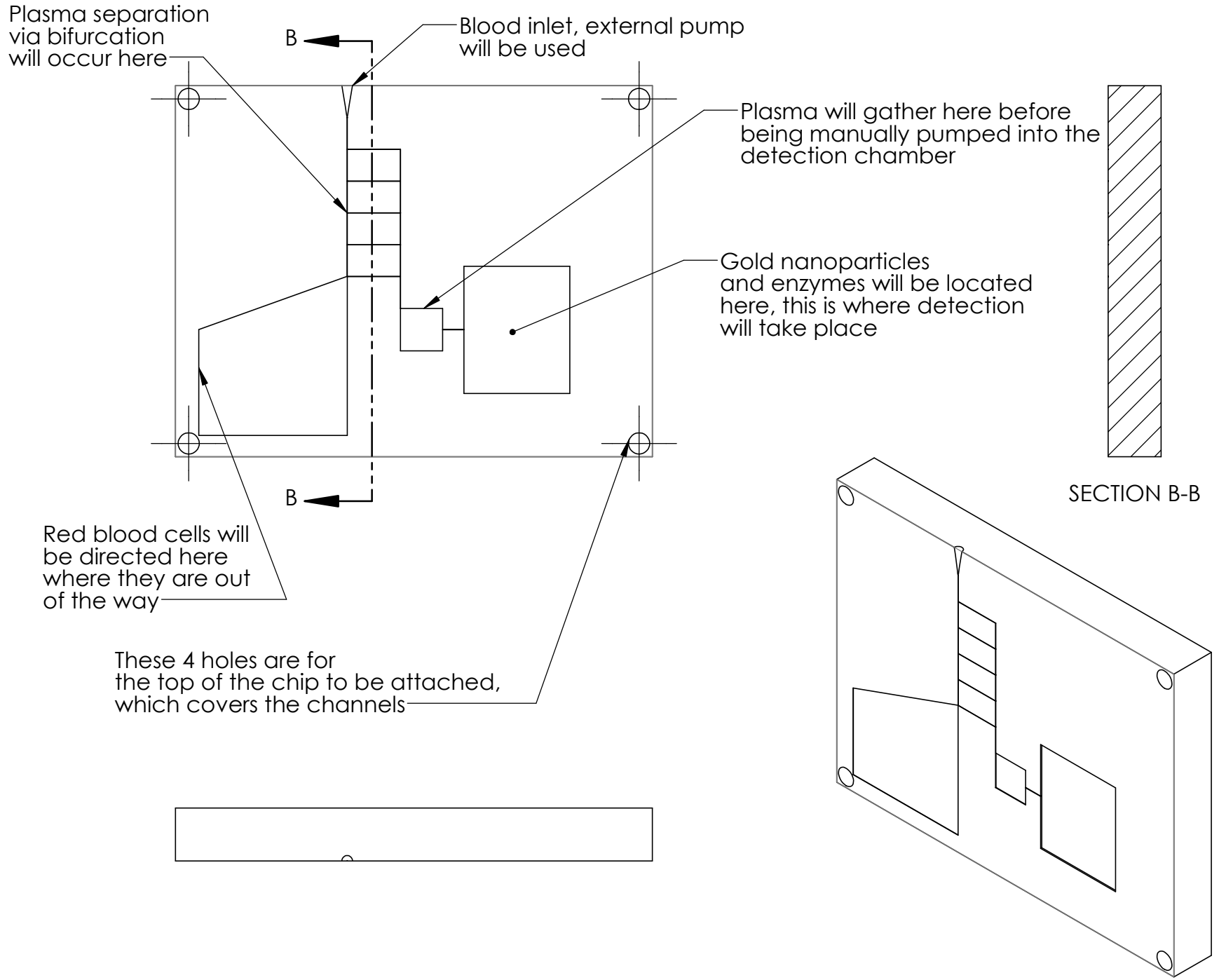
[13] Zhao, B., Cui, X., Ren, W. *et al.* "A Controllable and Integrated Pump-enabled Microfluidic Chip and Its Application in Droplets Generating." *Sci Rep* 7.11319(2017): 1-8.

Drawings:



The Ohio State University First Year Engineering	Dwg. Title: NANOLYSER Top	Inst.: Parke	Scale: 2:1	Dwg. No.: 2
	Drawn Bv: Team Y4	Hour: 12:40	Units: MMGS	Date: 3/5/20



The Ohio State University First Year Engineering	Dwg. Title: NANOLYSER Chip Bottom	Inst.: Parke	Scale: 2:1	Dwg. No.: 1
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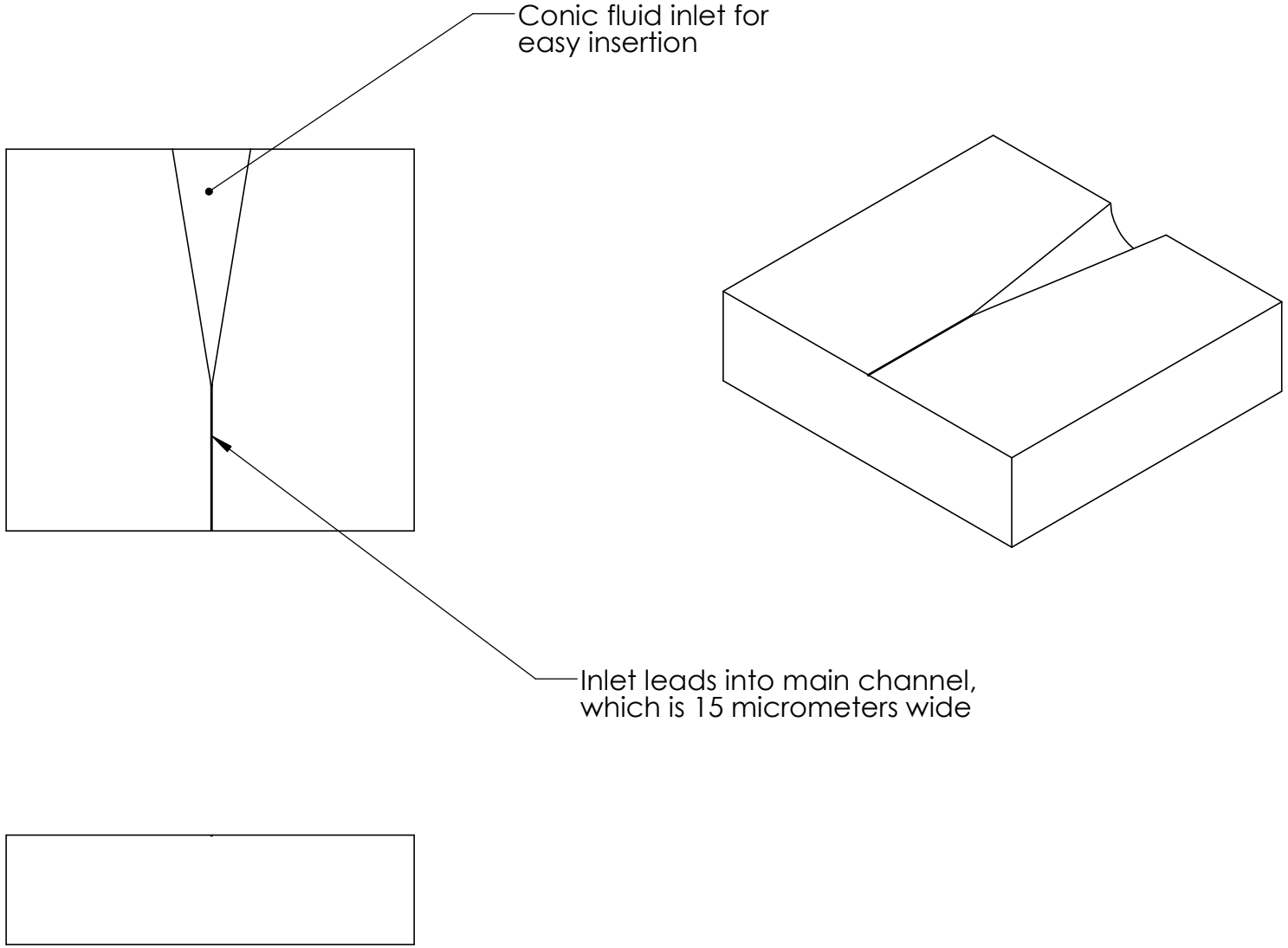

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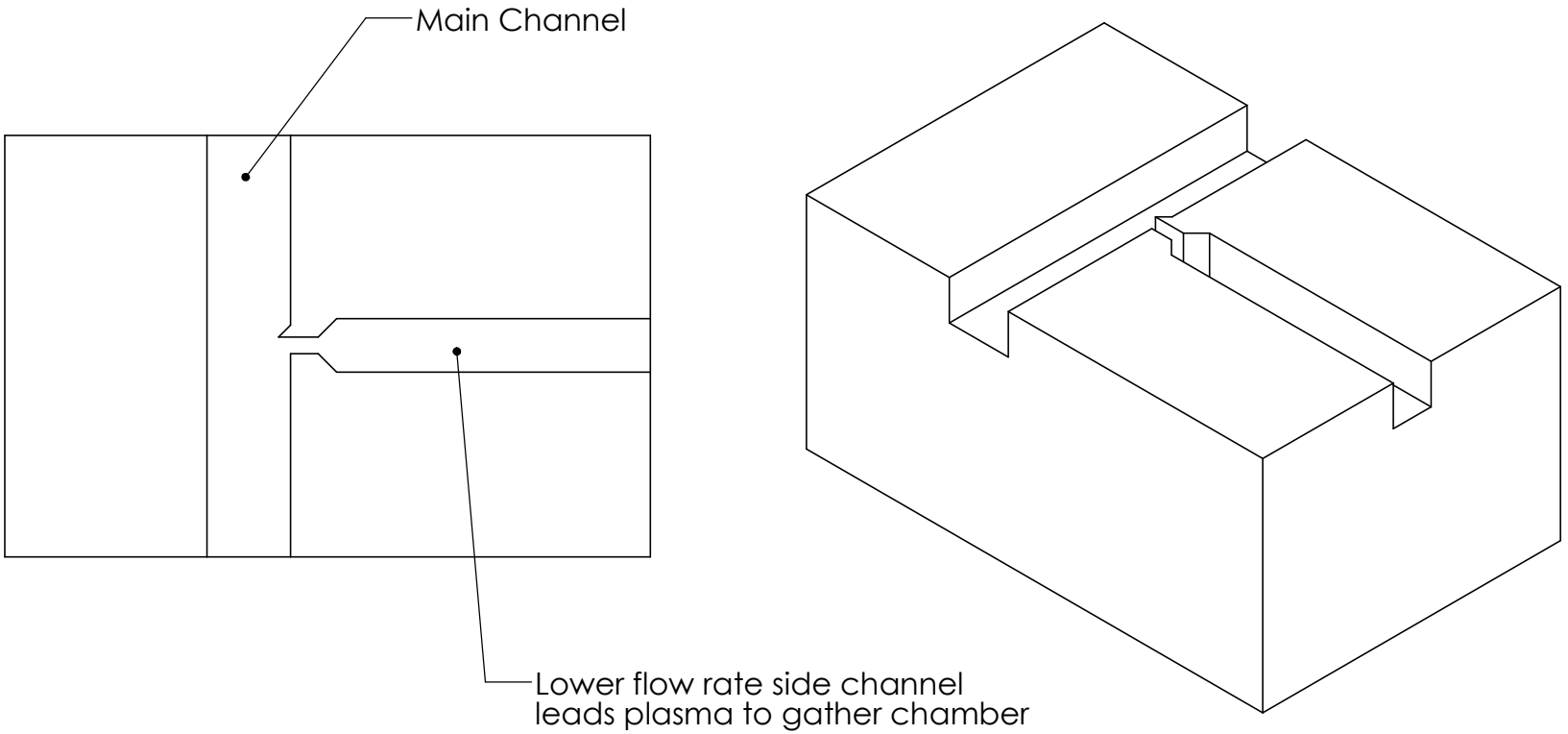
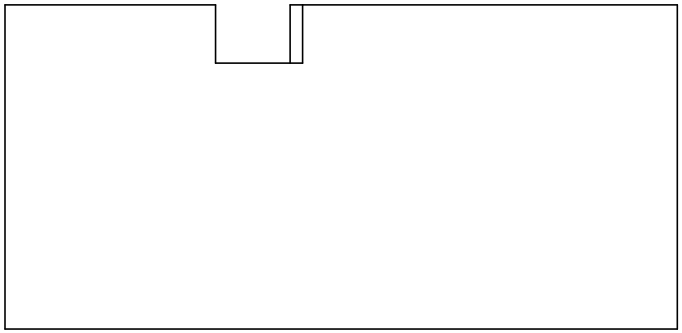
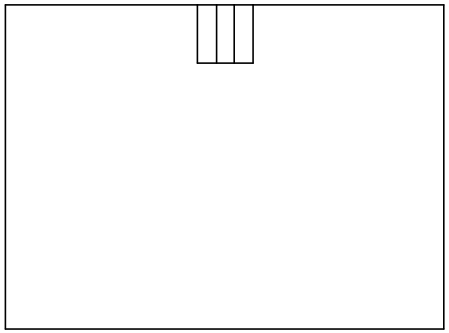
	Drawn By: Zachary Niswonger	Hour: 12:40	Units: MMGS	Date: 4/7/20







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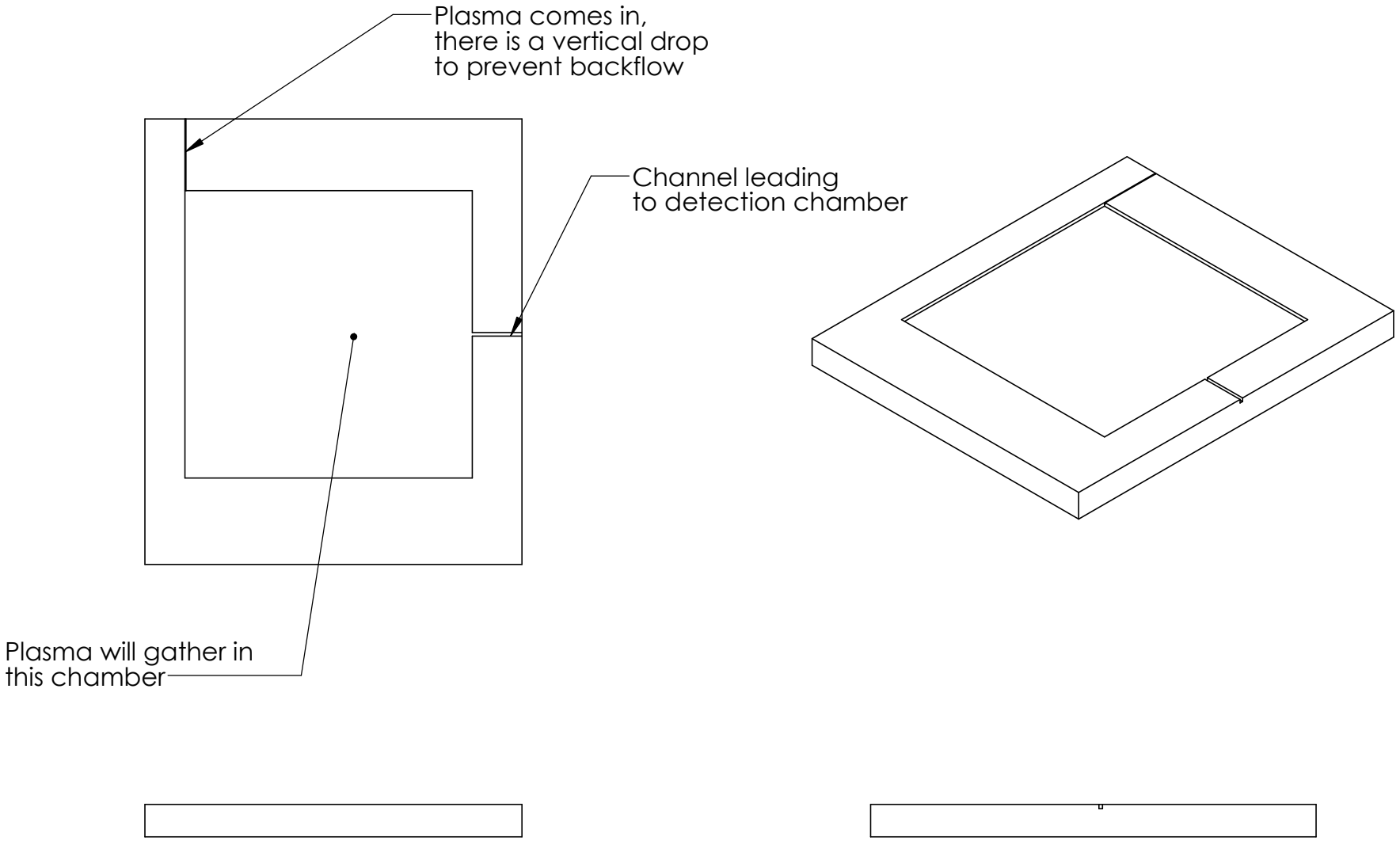

The Ohio State University First Year Engineering	Dwg. Title: NANOLYSER_Plasma_Chamber	Inst.: Parke	Scale: 12:1	Dwg. No.: 1
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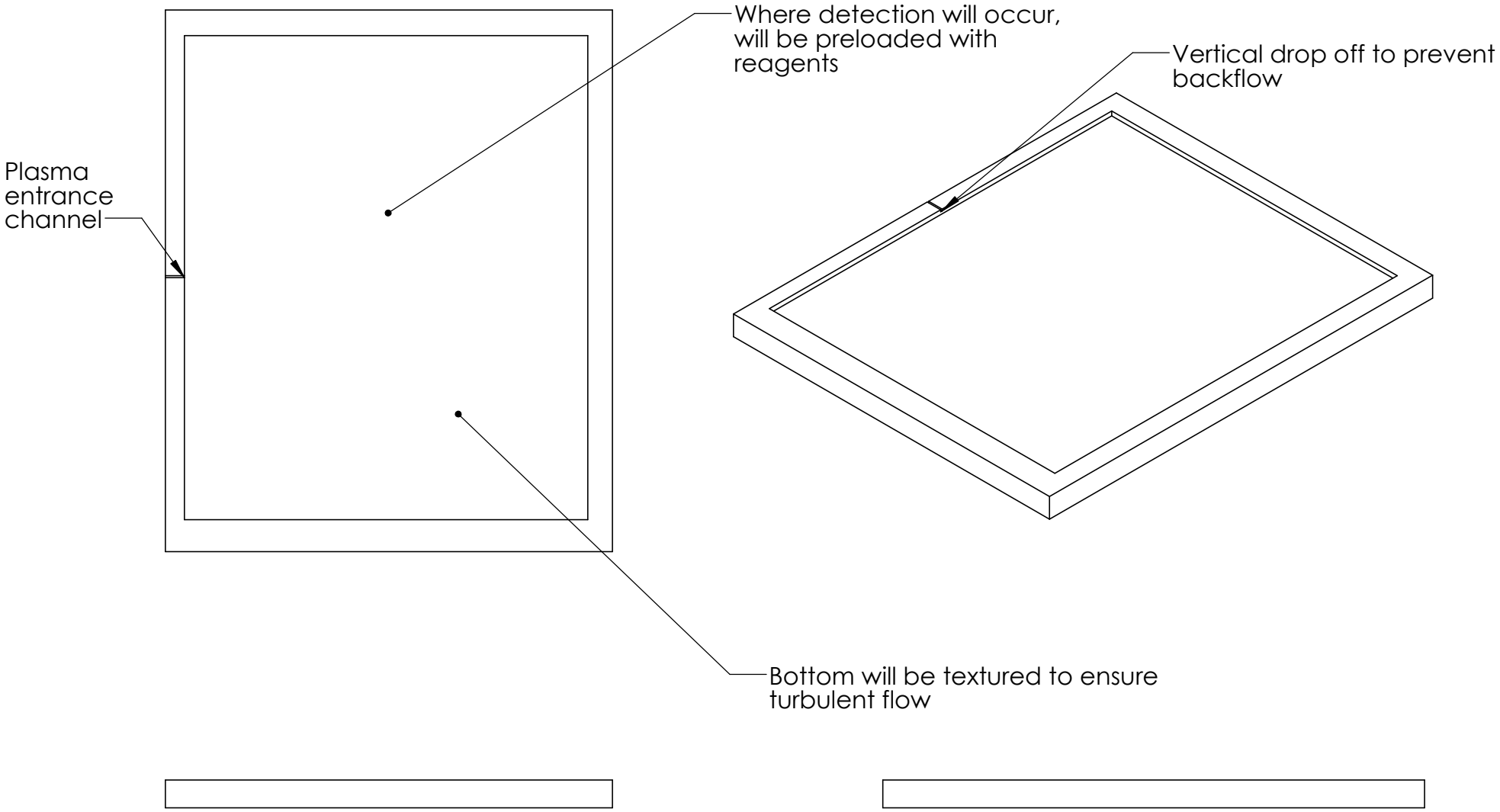

The Ohio State University First Year Engineering	Dwg. Title: NANOLYSER_Detection_Chamber	Inst.: Parke	Scale: 7:1	Dwg. No.: 1
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