Internet of Nano-Things in human cancer detection

Robert-Daniel Banu
University of Twente
P.O. Box 217, 7500AE Enschede
The Netherlands
r.d.banu@student.utwente.nl

ABSTRACT

One of the leading causes of death worldwide is cancer. This is in part due to the current screening methods that medical professionals have at their disposal, as they are not able to detect tumors that are minuscule. The earlier a cancer is detected, the higher the likelihood of the patient's survival. Would it be possible to create a more specific screening method, and what size tumors would it be able to find? As a solution to this problem, this paper envisioned an Internet of Nano-Things cancer screening technique, in which genetically engineered bacteria are inserted into the body of a testee, where they would colonize the tumor and release a fluorescent marker. This marker would be detected by a nano-device which would ping an internet getaway bracelet worn by the testee. Based on the weight and height of the patient, the amount of bacteria given to them, the first time of detection, and duration of pings, the procedure would be able to approximate the size of the tumor present in the testee. This paper found that the theoretical minimum detectable tumor size for such a technique varies between $40mg$ and $131mg$, depending on the patient's blood volume, with the patient being administered 121 000 bacteria.

Table 1. Symbols Legend

V_T	Tumor Volume (ml)
V_P	Plasma Volume (ml)
C_T	Concentrations of ZsGreen in the tumor ($\mu g \cdot ml^1$)
C_P	Concentrations of ZsGreen in the plasma ($\mu g \cdot ml^1$)
C_B	Tumor bacterial density ($CFU \cdot mg^1$)
K_v	Mass transfer rate constant (h^{-1})
K_e	Plasma clearance rate constant (h^{-1})
CFU	Colony forming units
CFU_{admin}	Bacteria number administered
m	ZsGreen production rate per bacterium ($fg \cdot CFU^{-1} \cdot h^{-1}$)
BMI	Body mass index
V_B	Blood volume
LSF	Lean-Scaled Factor
B_F	Female blood volume
B_M	Male blood volume
M_z	Minimum detectable ZsGreen $\mu g \cdot l^{-1}$

Keywords

Internet of Nano-Things, IoNT, bacteria, cancer detection, ZsGreen

1. INTRODUCTION

Cancer is the second most deadly malady, killing millions around the world every year[8]. Two ways of increasing cancer survival rates are improving early detection rates and bettering current surveillance methods. Here, surveillance refers to the monitoring of tumor recurrence through screening methods. Both of these methods could be advanced by creating a more sensitive and accurate cancer screening method.

Cancer screening methods are tests performed on individuals who are at a higher risk of cancer (e.g. people with family history of cancer or smokers), which attempt to detect tumors sooner, or to prevent any tumor-related complications [9]. Furthermore, these tests can be used for tumor surveillance, which is an important factor in the full recovery of a patient.

Some current screening techniques have tried measuring the natural tumor biomarkers of patients in order to diagnose early, but the techniques turned out to be underwhelming, as their accuracy (lack of errors) and specificity were below standard. Specificity, in the context of biomarker-based screening methods, refers to the ability of the test to correctly identify patients who do not have cancer [9].

Other screening techniques are based on imaging a part or parts of the body of a patient and looking for irregularities. Examples of such tests are tomographies, X-ray scans, and ultrasound imaging. These tests are often expensive (due to the machinery required) and take up a lot of medical personnel time, which limits the number of screenings a hospital can perform at a given time.

Then, the question is, can we come up with a cancer screening method which is both accurate and specific as well as scalable ? One such solution could be an Internet of Nano-Things network which incorporates tumor-colonizing bacteria. The Internet of Nano-Things is a network of interconnected (nano-)devices which communicate with each other and function independently of any human input.

33^{th} Twente Student Conference on IT July. 3^{rd}, 2020, Enschede, The Netherlands.

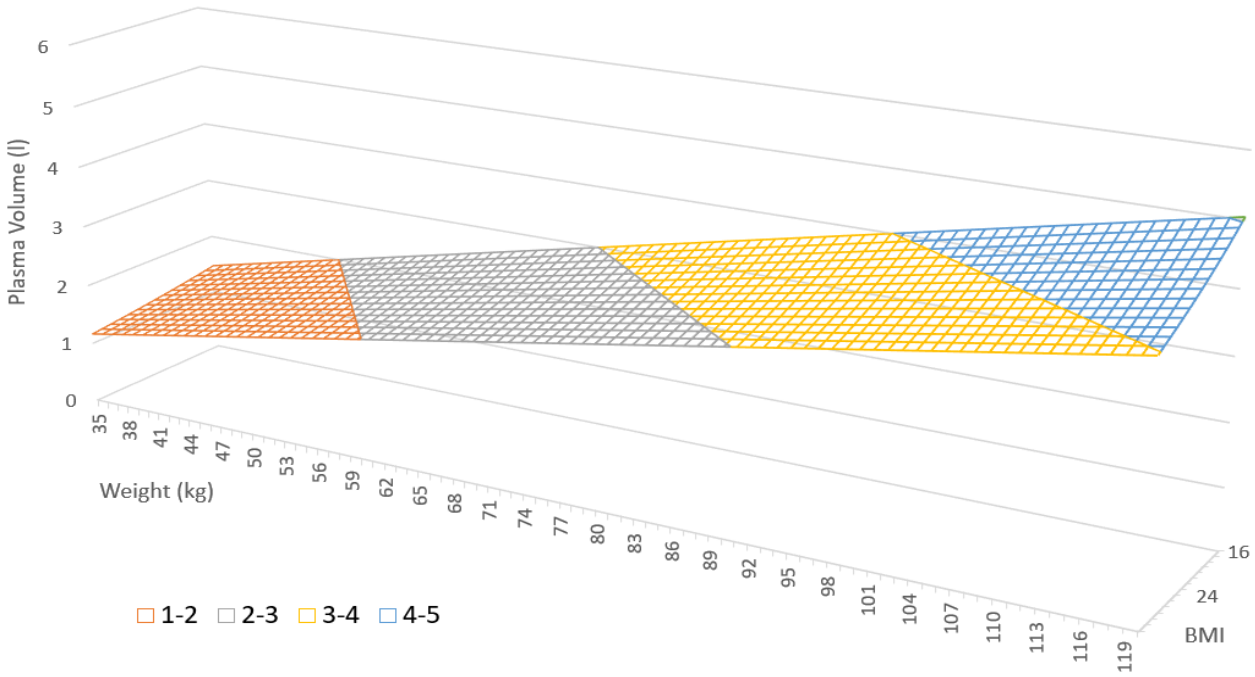

Figure 1. Graph showing the plasma volume based on BMI and weight of a female patient.

2. METHODOLOGY

2.1 Envisioned Procedure

This paper proposes an Internet of Nano-Things network which would combine tumor-colonizing bacteria, a patrol (nano-device), and an internet gateway bracelet in order to screen a patient for any possible tumors.

The bacteria chosen for this technique would be a non-pathogenic strain of Salmonella, made 10 000 times less toxic to living organisms due to a change in the bacteria's genes [2]. Furthermore, these bacteria would also be genetically engineered to produce and release ZsGreen when their quorum is reached. Quorum sensing is the ability of unicellular organisms to communicate with each other through release of microchemicals, enabling them to control their population size and other functions [6]. ZsGreen is a fluorescent marker protein, which in doses relevant to this procedure, is not harmful to humans, and is used a as a signal between the bacteria and the patrol.

The envisioned procedure sees the testee being injected with the aforementioned bacteria and a patrol. The testee would also be given a bracelet which would be paired with their cell phone, in order to connect the network to the internet, where it can send the results to the patient's doctor.

After the bacteria are introduced into the bloodstream, they would travel along the circulatory system and sense the biomarker of a possible tumor. At some point, through chemotaxis, the bacteria would follow the biomarker to the tumor [5]. Chemotaxis is simply the movement of an organism based on external chemical stimuli. The bacteria would reach the tumor and accumulate as colonies within it [7].

Once enough bacteria accumulated inside the tumor and are in the vicinity of one another, their quorum sensing would be activated, and they would start producing and releasing ZsGreen. This ZsGreen would first accumulate inside the tumor, and over time be released into the bloodstream through the vascular surfaces of the tumor (see **figure 2**).

The aforementioned patrol is a nano-device that would be making rounds along the bloodstream and test every hour for the concentration of ZsGreen present in the patient's plasma. This patrol would be powered through magnetic coupling [1], having the bracelet worn by the patient wirelessly charge the patrol's power supply. If the patrol reads a positive result in its hourly test, it would ping the bracelet, which would forward the ping to the main servers.

The program running on said servers would count the number of pings it has received, the time of first detection, and together with the height and weight of the patient and the number of bacteria administered, it would be able to approximate the size of the tumor of the patient and communicate it to the patient's doctor for further action.

This proposed procedure can be seen as having two distinct phases. In its First Phase, the bacteria enter the bloodstream through an injection and ride along the blood flow until, at some point, they reach any possible tumors through chemotaxis. The Second Phase starts when the quorum of the bacteria inside the tumor is activated and they start producing and releasing ZsGreen.

This paper focuses on this Second Phase and looks at the diffusion of ZsGreen into the bloodstream, measuring the moment at which it could be detected, as well as how long it could be detected for, depending on plasma volume, bacteria administered, and tumor size. For this, a simulation was built, which simulates the ZsGreen level inside the tumor and the plasma of the patient, and is modeled based on the diffusion model provided by Panteli et al. [7]. Furthermore, because the minimum detectable

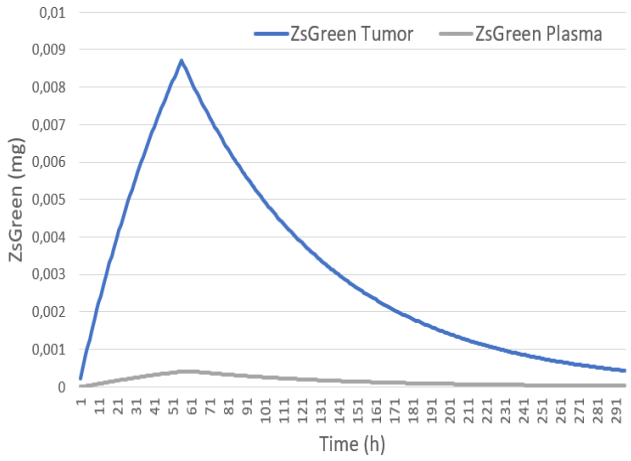

Figure 2. Level of ZsGreen in tumor and in plasma over time. Regardless of the tumor size, bacterial density, and plasma volume, the graph would have the same approximate shape.

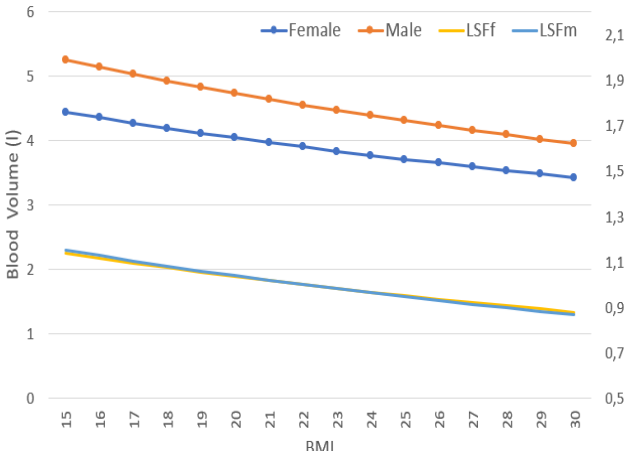

Figure 3. The blood volume and lean-scaled factor of a person weighing 65 kg is calculated based on different BMIs. The yellow and light blue lines are pegged on the secondary axis (right axis) and represent the lean-scaled factor for female patients (yellow eq.(5)) and for male patients (light blue eq.(6)). The dotted blue line is calculated using equation (8) for female patients and the red line is calculated using equation (9) for male patients, both pegged on the main axis.

Table 2. Variables values

V_P	1 - 5 l
C_B	0 - 486 CFU$\cdot mg^{-1}$
m	4.3 $fg \cdot CFU^{-1}$ (1 fg = 1 femtogram = $10^{-9}\mu$g)
K_V	0.0125 h^{-1}
K_e	0.259 h^{-1}
M_z	104.2$\cdot 10^{-6}$ $\mu g \cdot l^{-1}$

ZsGreen level depends directly on the plasma volume of the testee, a model for the calculation of plasma volumes was also created.

3. SIMULATION

3.1 ZsGreen diffusion model

The simulation performed looked specifically at the level of ZsGreen within the bloodstream. For this, a model for the ZsGreen diffusion from tumor to bloodstream was needed. Panteli et al. conducted an experiment in which they injected tumor bearing lab mice with the aforementioned bacteria and measured the ZsGreen levels present in their blood. The following equations, (1) and (2), are the result of Panteli et al.'s work, and will be the core of this simulation's ZsGreen diffusion model [7]. Please consider examining the abbreviations table (**table 1**) before reading further.

Firstly, the equations representing the amount of ZsGreen in the tumor:

$$V_T \frac{dC_T}{dt} = mC_B V_T - K_v(C_T - C_P)V_T \qquad (1)$$

This equation is a function which describes the concentrations of ZsGreen in the tumor (C_T), which changes with time $d(t)$. With each tick of the simulation (which represents one hour), ZsGreen in produced inside the tumor equal to the ZsGreen production rate per bacterium (m) multiplied by the tumor bacterial concentration (C_B) multiplied by the volume of the tumor (V_T). At the same time, an amount of ZsGreen equal to the product of V_T, the mass transfer constant(K_V) and the difference between C_T and plasma ZsGreen concentration (C_P), is diffused into the bloodstream.

Secondly, the equation representing the ZsGreen present in plasma is:

$$V_P \frac{dC_P}{dt} = K_v V_T(C_T - C_P) - K_e C_P V_P \qquad (2)$$

This equation describes the Concentration of ZsGreen in the plasma. The amount of ZsGreen explained in the previous paragraph $K_v V_T(C_T - C_P)$, is added to the total plasma fluoromarker ($V_P \frac{dC_P}{dt}$). The ZsGreen inside the plasma is cleared out at a rate equal to the total plasma volume (V_P) multiplied by the plasma clearance rate constant (K_e) further multiplied by C_P. So, with each tick of the simulation, the total ZsGreen in plasma increases by the fluoromarker amount diffused from the tumor and is decreased by the plasma clearance rate.

Lastly, in the experiment performed by Panteli ., only 53% of the administered bacteria ended up producing ZsGreen [7], ergo the bacterial density (C_B) can be calculated given a tumor size and the amount of administered CFU (CFU_{admi}) as such:

$$C_B = \frac{CFU_{admi}}{V_T} \cdot 53\% \qquad (3)$$

And conversely, to obtain the administered CFU required to obtain a desired C_B :

$$CFU_{req} = V_T \cdot C_B \cdot \frac{100}{53} \qquad (4)$$

3.2 Plasma model

The plasma volume of a person can be calculated as a percentage of their blood volume. The total blood volume of a person can be be calculated simply by multiplying the patient's weight by the blood volume index, which is 65 $ml \cdot kg^{-1}$ for female patients and 70 $ml \cdot kg^{-1}$ for male patients. This method produces a fairly accurate approximation of the patient's blood volume, but, towards

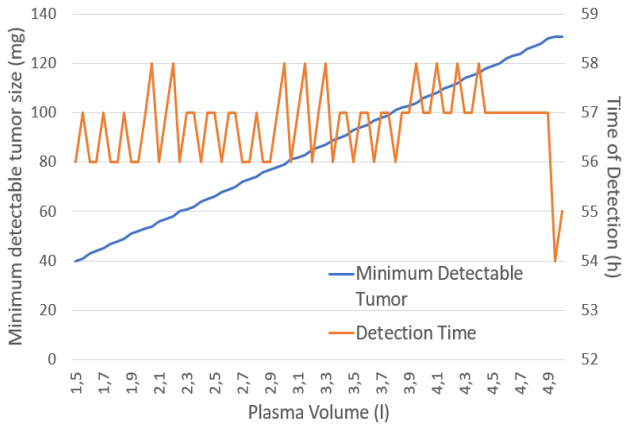

Figure 4. Minimum detectable tumor size based on the plasma volume assuming 486 $CFU \cdot mg^{-1}$. The main axis is the size of the minimum detectable tumor for a given plasma volume (blue line) and the secondary axis represents the time of its detection (orange line).

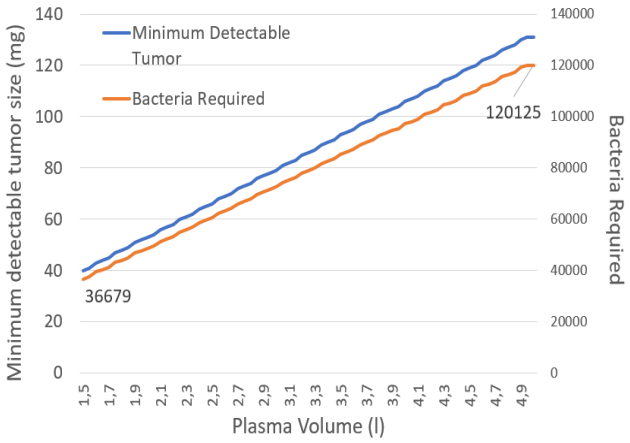

Figure 5. Minimum detectable cancer based on the plasma volume assuming 486 $CFU \cdot mg^{-1}$. The main axis is the size of the minimum detectable tumor for a given plasma (blue line) and the secondary axis represents the minimum amount of bacteria administered to the testee for the tumor to be detected (orange line).

the heavier end of the spectrum, the method has been found to overestimate plasma volume. Ergo, this paper uses the method proposed by John H. P. Friesen, namely, using the lean-scaled weight of a patient in calculating their blood volume [4]. The lean-scaled weight is a weight scalar, used in fields such as drug dosage, equal to the patient's weight multiplied by the lean-scaled correction factor.

The lean-scaled correction factor for female patients is :

$$LSF_F = \frac{14148}{8780 + 244BMI} \tag{5}$$

And for male patients it is :

$$LSF_M = \frac{11432}{6680 + 216 \cdot BMI} \tag{6}$$

In both equations BMI is the Body Mass Index, which is calculated as :

$$BMI = \frac{Weight}{Height^2} \tag{7}$$

In order to obtain the V_B, the lean-scaled correction factor has to be multiplied by the patient's weight and the Blood Volume Index, which is 65/70 $ml \cdot kg^{-1}$ for female/male patients [4]. Thus the blood volume of the patient being calculated as follows :

$$B_F = LSF_F \cdot Weight \cdot 65 \tag{8}$$

$$B_M = LSF_M \cdot Weight \cdot 70 \tag{9}$$

And lastly, the plasma volume is equal to 58% of the blood volume [3]:

$$V_P = \frac{58}{100} \cdot V_B \tag{10}$$

3.3 Set-up

The core of the simulation was a function which, given a number of bacteria administered, a tumor size, and a plasma volume, returns a true or false result based on whether or not the given tumor is detectable by the patrol at the given CFU_{admin} and V_P. Using this core function, other methods were created, each returning a different result. One found the minimum detectable tumor for a given C_B and varying V_P, another one found the minimum C_B for a given V_T with varying V_P. Furthermore, methods calculating the time in plasma and time of detection were also written. All these functions were written in the Kotlin programming language .

Table 2 contains the determined values of some variables from equations (1) and (2). V_P was calculated using the plasma model previously discussed and its values are elaborated on in the Results section of this paper. All other values were taken from Panteli .'s study [7].

The envisioned system's patrol would be able to detect ZsGreen in plasma at a concentration of 1 μmol per liter. This is the same minimum detectable concentration used by Panteli . [7], who performed a blood test in order to determine the plasma ZsGreen concentration. The molar mass of ZsGreen is 104.2 g·mol^{-1}, making the minimum detectable ZsGreen 104.2·10^{-6} μg per liter, or 104.2 ·10^{-9} μg per millilitre.

Additional consideration has to be given to the bacterial density (C_B). In their study, Panteli et al. mention that only 53% of bacteria inside the tumor produced ZsGreen, and that bacteria colonized tumors at an average density of 486 CFU·mg^{-1}. It is unclear if the number 486 represents all bacteria, or just the ZsGreen-producing bacteria. This simulation interpreted 486 to be the amount of ZsGreen-producing bacteria, and set it as the maximum achievable C_B. If this assumption is wrong, all results achieved in the simulation would be different. These alternative results are discussed in the Discussion subsection.

Lastly, Panteli et al.'s experiment had the bacteria produce ZsGreen for 56 hours, Thus, this simulation also has the bacteria produce ZsGreen for 56 hours.

4. RESULTS

4.1 Plasma Volume

The BMI (**equation** (7)) of patients was calculated for

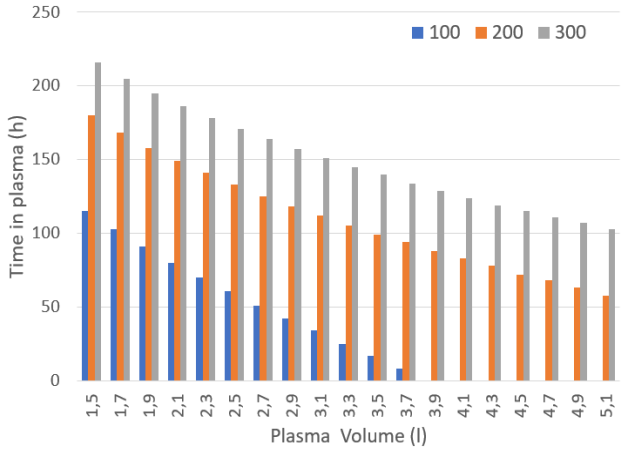

Figure 6. Length of time in which the ZsGreen level is detectable in plasma for a tumor of $100mg$, $200mg$ and $300mg$, with 120 000 CFU administered to the testee. The $100mg$ tumor is not detectable if the plasma level is above 3.8 l.

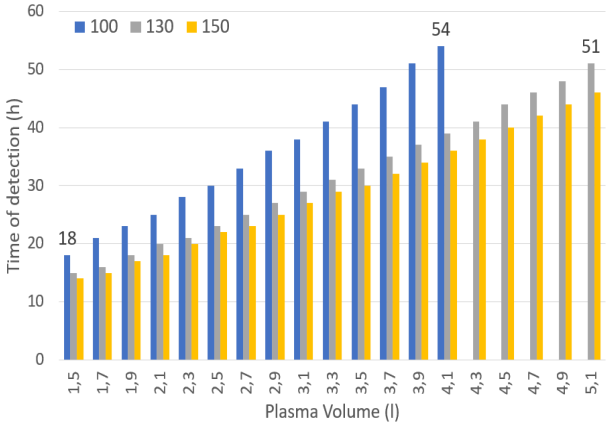

Figure 7. Detection time for a tumor of $300mg$, varying with plasma volume. The graph has three series: 100 000, 130 000 and 150 000 bacteria administered to the testee.

heights between $1,3m$ and $2.3m$ and weights between $35kg$ and $150kg$, resulting in BMI values between 5 and 90. For ease of calculation, the extreme cases were ignored (BMI < 15 and BMI > 30), so BMI between 15 and 30 was used in calculating the total plasma volume of female and male patients using equations (8) and (9) respectively. In **figure 1** the plasma variation based on BMI and weight for a female patient can be seen. It can be observed that the plasma volume varies inversely with BMI and directly with weight. The calculated plasma volume values were between 1 and 5 liters.

Likewise, the blood volume and lean-scaled factor variation with BMI of a female patient and a male patient, both weighing 65 kg, was calculated and is displayed in **figure 3**. It can be observed that the blood volumes go down with increased BMI, which is expected because the lean-scaled factor also decreases with increased BMI, and the lean-scaled factor is directly proportional to the blood volume. The difference between the blood volume of male and female patients is due to the difference in blood volume index, which is 65 $ml \cdot kg^{-1}$ for female patients and 70 $ml \cdot kg^{-1}$ for male patients. It can also be observed that the lean-scaled factor's difference between the two sexes is relatively small.

4.2 Minimum detectable tumor

The maximum amount of bacteria density per mg of tumor, for the purpose of this study, is considered to be 486 CFU$\cdot mg^{-1}$. It follow, that the minimum detectable cancer will be achieved with $C_B = 486$, as the higher the bacterial density is, the smaller the minimum detectable cancer. The plasma volume of the patient has a direct relation to the minimum detectable cancer. The more plasma a patient has, the larger the minimum detectable tumor. This is because the minimum detectable ZsGreen is 1 μmol **per liter**, so more net ZsGreen is needed for detection. Moreover, the ZsGreen clearance rate is dependant on the total plasma volume($K_e C_P V_P$).

Figure 4 shows the minimum detectable tumor depending on plasma at maximum $C_B = 486 CFU \cdot mg^{-1}$ on the main axis. The minimum detectable tumor for $1,5l$ of plasma is $40mg$ and $131mg$ for $5l$ of plasma. The minimum detectable tumor, as predicted, increases with the plasma volume. It increases on average with $1,3mg$ for every $0,05l$ increase in plasma. On the secondary axis, the time of detection of each minimum tumor is shown. It can be observed that the time of detection in this graph varies between $54h$ and $58h$. This is because the minimum detectable tumor for a given plasma volume is an edge case, which happens towards the end of the $56h$ production cycle when the ZsGreen amount being diffused is close to or at its largest. For the tumor to be detected, the fluoromarker diffused from the tumor has to overcome the plasma clearance rate.

Figure 5 again shows the minimum cancer detectable with maximum bacterial density on the main axis. The second axis shows the minimum amount of bacteria required for said minimum tumor to be detected. It can be observed that at least 120 125 bacteria are required for $5l$ of plasma and 36 679 CFU for $1,5l$ of plasma. Towards the lower end of the plasma volume spectrum, having more bacteria than required does not influence the minimum detectable tumor size, nor the time of detection or time in plasma, as the extra bacteria simply would not fit into the tumor and would not trigger their quorum to start producing ZsGreen.

4.3 Time of detection

The time of detection is the time at which the patrol first detects the ZsGreen in the plasma. As explained in the previous section, 121 000 bacteria would be necessary for the minimum detectable tumor for all plasma volumes. As long as the tumor size is large enough (C_B does not surpass 486 $CFU \cdot mg^{-1}$), the time of detection would decrease with an increased number of bacteria administered.

For example, in **figure 7** the simulated tumor size is $300mg$ and its time of detection is recorded for three different amounts of bacteria administered: 100 000 ($C_B = 177 \cdot CFU \cdot mg^{-1}$), 130 000 (230 CFU$\cdot mg^{-1}$) and 150 000 (265 CFU$\cdot mg^{-1}$). So, generally, the time of detection goes down with less plasma volume and more CFU administered, although it can reach a minimum time of detection when he C_B reaches 486 $CFU \cdot mg^{-1}$.

4.4 Time in Plasma

As previously mentioned, the ZsGreen accumulates in the tumor and it slowly diffuses into the bloodstream (see **fig-**

ure 2). Moreover, each tick of the simulation, ZsGreen is cleared from plasma, meaning that over time the ZsGreen will completely diffuse into the blood and be cleared out. The time in plasma is the amount of time the ZsGreen level in plasma is above the minimum detectable level.

Figure 6 shows the time in plasma for three different tumor sizes: $100mg$, $200mg$ and $300mg$. It can be remarked that the time in plasma is shorter with increased plasma volume. This is to be expected because the more plasma there is, the more ZsGreen is cleared out every hour. Moreover, the time in plasma goes up with the amount of bacteria administered, again, as long as C_B has not reached 486 $CFU \cdot mg^{-1}$.

5. DISCUSSION

This research analyzes the production and diffusion of ZsGreen by tumor colonizing bacteria and it looks at minimum detectable tumor sizes, their detection time and the amount of time the ZsGreeen produced by the bacteria stay in the plasma, all varying with the plasma volume of the testee.

Using the approximated plasma volume of the patient, the number of bacteria administered, the time of detection, and the time in plasma, the procedure can approximate the tumor size of the testee. For example, a female patient who weighs $56kg$ and is $1.6m$ tall has a BMI of 22 and $2l$ of plasma. She is administered a dose of 120 000 CFU, together with the patrol, and is given the gateway bracelet to wear. She pairs the bracelet with her phone so it has an internet connection. 26 hours after the procedure, the clinic which administered the test receives the first ping coming from the IoNT network. For 86 hours, the bracelet pings the clinic hourly, after which it stops. So, detection time is 26 and time in plasma is 86. Now that all the variables are present, the doctors would be able to determine that the patient has a tumor weighing $100mg$ and can contact them for further actions. This novel cancer-screening method could offer accurate results with minimal patient discomfort and medical resources required.

In order to run the simulation, multiple assumptions had to be made regarding the variables involved. The simulation assumed that the modeling performed by Panteli et al.'s in their experiment performed on lab mice would apply to humans. Constant values such as the mass transfer rate, the plasma clearance rate, the ZsGreen production rate, and bacterial density were all sourced from their study [7]. Although these values most likely would not be directly applicable to human subjects, this paper serves as an inkling of what performance such a cancer screening system would have. Moreover, Panteli et al. mention that using their results, their system would be able to detect tumors as small as $124mg$, which is similar to the results of the research conducted for this paper. Furthermore, a nano-device which can detect ZsGreen inside the plasma has not yet been invented. However, the invention and production of such a device in coming years is plausible.

Returning back to the aforementioned point of confusion, if Panteli et al. meant 486 to be the overall bacterial density, then the maximum ZsGreen producing bacterial concentration for the simulation would be 258 CFU·mg^1. This would make the minimum detectable tumor for $1,5l$ of plasma be $80mg$ and for $5l$ of plasma, $264mg$ (increasing by approximately $2,5mg$ for every $0,05l$). Moreover, the required bacteria would also be halved.

6. CONCLUSION & FUTURE WORKS

With regard to future works relating to the topic of this paper, the First Phase of the procedure could be analysed. The chemotaxis of the bacteria could be modeled inside the human body to see how long it would take for them to colonize any tumors. The chomatxis has already been modeled inside a free three dimensional space [5], but the behavior of the bacteria would be different inside a human body, as they would first have to travel through the bloodstream and exit it when they detect any biomarkers originating from the tumor.

In conclusion, the procedure presented in this paper could potentially be used as a powerful tool in humanity's fight against cancer. The bacteria colonize tumors with high accuracy, making the procedure highly specific (the ZsGreen plasma concentration would only reach detectable levels if the bacteria colonize a tumor). Moreover, this procedure would only require a single visit to a medical professional and would not require large expensive machines. Thus, the procedure would be scalable, and could be made available to virtually anyone. Furthermore, is also suitable to be used as a part of surveillance, in order to increase patient recovery rates.

7. REFERENCES

[1] Wireless energy delivery and data communication for biomedical sensors and implantable devices. *Bioengineering, Proceedings of the Northeast Conference*, pages 6–7, 2009.

[2] C. Clairmont, K. Lee, J. Pike, M. Ittensohn, K. Low, J. Pawelek, D. Bermudes, S. Brecher, D. Margitich, J. Turnier, Z. Li, X. Luo, I. King, and L. Zheng. Biodistribution and Genetic Stability of the Novel Antitumor Agent VNP20009, a Genetically Modified Strain of Salmonella typhimurium . *The Journal of Infectious Diseases*, 181(6):1996–2002, 2000.

[3] J. Feher. Regulation of Arterial Pressure. *Quantitative Human Physiology*, pages 538–548, 2012.

[4] J. H. Friesen. Lean-scaled weight can be used to estimate blood volume for obese patients. *Canadian Journal of Anesthesia*, 61(11):1059–1060, 2014.

[5] C. Lo, K. Bhardwaj, and R. Marculescu. An autonomous and adaptive bacteria-based drug delivery system. *Proceedings of the 3rd ACM International Conference on Nanoscale Computing and Communication, ACM NANOCOM 2016*, 2016.

[6] P. Melke, P. Sahlin, A. Levchenko, and H. Jönsson. A cell-based model for quorum sensing in heterogeneous bacterial colonies. *PLoS Computational Biology*, 6(6):1–13, 2010.

[7] J. T. Panteli, N. V. Dessel, and N. S. Forbes. Detection of tumors with fl uoromarker-releasing bacteria. 149:137–149, 2020.

[8] M. Roser and H. Ritchie. Cancer. *Our World in Data*, 2020. https://ourworldindata.org/cancer.

[9] J. D. Schiffman, P. G. Fisher, and P. Gibbs. Early Detection of Cancer: Past, Present, and Future. *American Society of Clinical Oncology Educational Book*, (35):57–65, 2015.