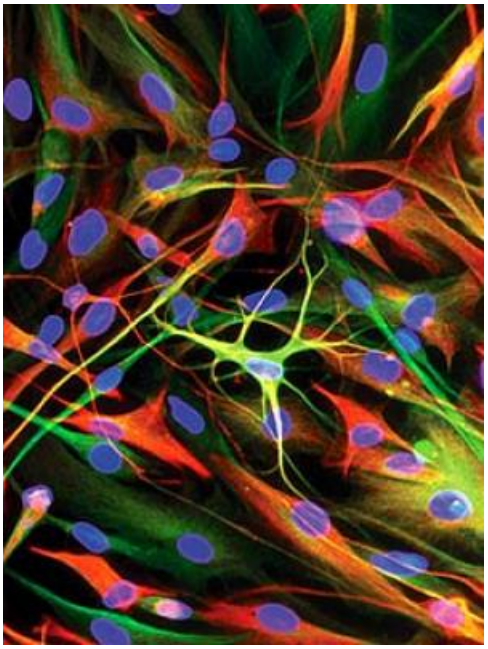
Evolution of a cancer cell population in a tissue

Federica Spiriti



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Introduction

The mathematical analysis discussed in this report focuses on a model representing the evolution of a population of cells in a tissue, in analogy with the classical model for bacterial growth in a chemostat. The basis of this study is the formulation of a model which describes the dynamics of cancer cells taking into account their metabolism and the pH component. The idea which has motivated this work is the intention hereinafter to compare how two population of cells, typically healthy and cancer cells, evolve and interact in the same environment, consuming glucose and oxygen.
The presented analysis is based on a two dimensional dynamical system which describes the behavior of cancer cells in a substrate of glucose. In the first section we introduce the model, defining the variables and the set of parameters. Then we briefly present the higher dimension system that models the interaction of two type of cells lying in the same tissue, in presence of oxygen and glucose. The main part of the report is devoted to a mathematical analysis of one population model: we start from a qualitative study to determine nullclines and equilibria, then we discuss the stability of the steady states and we present the system behavior with respect to some parameters that have a particular biological meaning. We conclude with an interesting interpretation of the mathematical results which could be useful for further study in the same field.
The discussed analysis is supported by the use of the software XPP-Aut due to Bard Ermentrout (available on http://www.pitt.edu/~phase/) which allows a good graphical approach for simulating, animating, and analyzing dynamical systems.

1 The model

The evolution of a population of cells in a tissue can be described in analogy with the classical model for bacterial growth in a chemostat. A chemostat is a device for harvesting bacteria (Fig 1): a stock solution of nutrient is pumped at some fixed rate into a chamber where the bacteria are being cultivated. An outflow valve allows the growth medium to leave at the same rate, so that the volume of the culture remains constant. A system with two ordinary differential equations describes how the microorganisms reproduce at the expense of nutrient consumption.

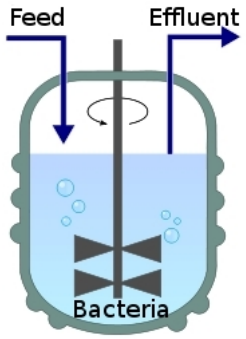

Figure 1: *Stirred bioreactor operated as a chemostat, with a continuous inflow (the feed) and outflow (the effluent). The rate of medium flow is controlled to keep the culture volume constant.*

In the same way, we consider one type of cells, cancer cells, into a culture chamber (the tissue), that consume nutrients coming in through arteries. We assume to have one substrate, typically glucose, since we know that cancer cells prefer to use glucose more then oxygen in their growth process. In order to maintain a convenient population level, we suppose that some cells leave the tissue and that the volume of the culture remains constant.
Let x denote the cells density and S the substrate concentration. The model describing their dynamics is the following

$$\dot{x} = -\alpha D x - m x + \mu(S,h)\, x \tag{1}$$

$$\dot{S} = D S_0 - D S - \frac{1}{\eta}\, \mu(S,h)\, x. \tag{2}$$

First of all we can assert that the way x changes inside the culture chamber depends on three main factors: the growing capability of cancer cells, their metabolic capacity, the number of cells that flow out through veins and the natural cell death. It is obvious that the growth function gives a positive contribution while the other two terms are negative. Let us discuss the different parameters:

- αD represents the rate of cells which leave the tissue. We assume that $\alpha \simeq 0$. D is a constant related to the intake/output blood flow.
- m is a parameter which represents the rate of death for unit time.
- μ is a function which describes the growth process of cancer cells. It depends on two factors:
  - the concentration of glucose;
  - the concentration of protons or pH level.

The hydrogen-ion concentration $[H^+]$ is described by the function $h = h(x)$ that we define as

$$h(x) := h_0 + \gamma x$$

where $\gamma \in \mathbf{R}^+$ denotes the number of hydrogen-ions in cancer cells and h_0 represents the concentration of protons in the absence of cancer cells. It is related to the pH value of the healthy tissue.
Let us remark that, in mathematical terms, the potential hydrogen or pH[1] is defined as the negative common logarithm of the concentration of hydrogen ions $[H^+]$ in moles/litre: $pH = -log_{10}[H^+]$.
The growth function μ is strictly related to the function h. Normally if h has a big value, the blood acidity is high and this makes healthy cell life difficult. Cancer cells can live in acidity solutions more easily then normal cells. In this model, we define the growth function as

$$\mu(S, h) := \frac{1}{k^2 + h^2} \left(\frac{lS}{k' + S} \right),$$

where $k, k', l \in \mathbf{R}^+$.
With regard to the second equation, it represents the rate of change of glucose in the culture chamber. The positive term DS_0 is due to the replenishment from blood coming in through arteries. The first negative term $-DS$ depends from the depletion due to the outflow of nutrient through veins. The last one is related to the part of nutrient solution consumed by cells in their growth process.
The following table lists the parameters we have introduced in our system:

Quantity	*Symbol*
Rate of cells which leave the tissue	α
Cell death rate	m
Intake/output blood flow rate	D
Glucose concentration in reservoir	S_0
Yield constant	η

[1]The pH scale is from 0 to 14 with 7.0 being neutral: anything above 7.0 is alkaline, anything below 7.0 is acid. Optimally, we want the fluids in our body to have a neutral or $7.0 - 7.2$ pH level.

1.1 Model with two population of cells

The evolution of cancer cells interacting with healthy cells in a tissue with two substrates, glucose and oxygen, is described by a dynamical system of four ordinary differential equations. This model is due to the collaboration between Jean-Luc Gouzé and Madalena Chaves (INRIA-Comore) and Frédéric Dayan (UNS-LJAD).
Let x_1, x_2 be respectively normal and cancer cells and S_1, S_2 glucose and oxygen concentration. The model is defined below.

$$\dot{x}_1 = -\alpha_1 D x_1 - m_1 x_1 + \mu_1(S_1, S_2, h)\, x_1 \quad (3)$$

$$\dot{x}_2 = -\alpha_2 D x_2 - m_2 x_2 + \mu_2(S_1, S_2, h)\, x_2 \quad (4)$$

$$\dot{S}_1 = DS_1^0 - DS_1 - \frac{1}{\eta_1^1}\,\mu_1(S_1, S_2, h)\, x_1 - \frac{1}{\eta_1^2}\,\mu_2(S_1, S_2, h)\, x_2 \quad (5)$$

$$\dot{S}_2 = DS_2^0 - DS_2 - \frac{1}{\eta_2^1}\,\mu_1(S_1, S_2, h)\, x_1 - \frac{1}{\eta_2^2}\,\mu_2(S_1, S_2, h)\, x_2 \quad (6)$$

The idea behind each equation is an extension of the discussion made in the previous section. The dynamics of the cell populations depends on the growing capability of cells - here function of both cell populations and substrates - their metabolism, the number of cells that flow out through veins and the natural cell death. The differential equations describing the evolution of the nutrients concentration are similar to equation (2) but clearly take into account the contribution of both populations in consuming substrate.
The model is conceptually simple but the big number of parameters and the totally absence of a priori information on their magnitude makes the analysis more complicated. The study of one population behavior could give a hint for the choice of the parameters and should be interesting for further studies.

2 Qualitative analysis

2.1 Nullclines

The first goal of qualitative analysis is to find the fixed points of the system that are solutions of the following problem

$$\begin{cases} -\alpha D x - m x + \mu(S, h)\, x &= 0 \\ DS_0 - DS - \frac{1}{\eta}\,\mu(S, h)\, x &= 0. \end{cases}$$

It is obvious that one steady state is given by $x = 0$, $S = S_0$. The corresponding function h is given by h_0 and represents the concentration of protons in the equilibrium state, $h = h_0 = 10^{-7.4}$.
We remark that the domain of our system is $x \geq 0$ and $S \geq 0$, since it is the only interesting region from a biological point of view. Sometime we take

into account a larger domain for completeness of the mathematical analysis. In order to obtain more information about equilibria and vector fields, it is useful to plot the nullclines, curves in the plane along which the rate of change of a particular variable is zero. The usefulness of these curves is that they break up the plane into regions along which the derivatives of each variable have a constant sign. Thus, the general direction of the flow is easy to determine. Furthermore, the intersections of the nullclines are the fixed points of the system.
In our case the x-nullcline and the S-nullcline are respectively solutions of the equations $\dot{x} = 0$ and $\dot{S} = 0$.

x-nullcline

$$(-\alpha D - m + \mu(S, h))\ x = 0. \tag{7}$$

One solution of equation (7) is clearly the line $x = 0$ and the other one is the solution of

$$-\alpha D - m + \frac{1}{k^2 + h^2}\left(\frac{lS}{k' + S}\right) = 0.$$

Then we have

$$S = \frac{(\alpha D + m)(k^2 + h^2)\, k'}{l - (\alpha D + m)(k^2 + h^2)}.$$

In order to well define the x-nullcline, we need to understand the behavior of $S = S(x)$. Since we know the expression of h as function of x, simply $h(x) = h_0 + \gamma x$, we can consider $S = S(h)$, without complicating the above formula and then we derive from that the behavior of S as function of x.
We have

$$S(h) = \frac{C(k^2 + h^2)\, k'}{l - C(k^2 + h^2)}$$

where $C = \alpha D + m$, $C > 0$. The analysis of function S is synthesized in few simple steps:

1. **Value in 0.** $S(0) = \frac{Ck'k^2}{l - Ck^2}$.

2. **Study of sign.** The sign of the function depends on the choice of the parameters. If we take the values such that $l - Ck^2 > 0$ then we have two vertical asymptotes

$$h_2 = -\sqrt{\frac{l - Ck^2}{C}} \qquad h_1 = \sqrt{\frac{l - Ck^2}{C}}. \tag{8}$$

The resulting function is shown in Fig 2. If we choose the parameters such that $l - Ck^2 < 0$, we have a function always negative as shown in Fig 3.

3. Limits.

$$\lim_{h\to\pm\infty} \frac{C(k^2+h^2)\,k'}{l-C(k^2+h^2)} = -k' < 0,$$

then we have an horizontal asymptote in the negative half-plane.

$$\lim_{h\to h_1^{\mp}} S(h) = \pm\infty \qquad \lim_{h\to h_2^{\mp}} S(h) = \mp\infty.$$

4. Derivatives.

$$S'(h) = \frac{2Ck'h(l-Ck^2-Ch^2)+Ck'(h^2+k^2)2Ch}{(l-C(k^2+h^2))^2} = \frac{2Ck'lh}{(l-C(k^2+h^2))^2}$$

From the above result, for $h > 0$ we have $S'(h) > 0$ so that the function is increasing, for $h < 0$ the function S in decreasing and there is a minimum for $h = 0$.

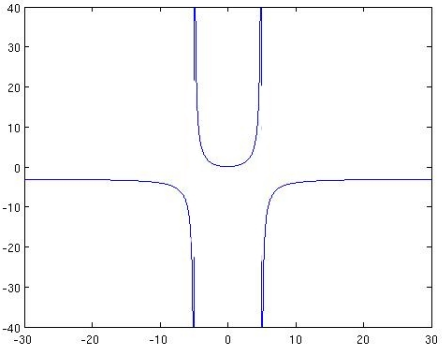

Figure 2: *x-nullcline in the case* $l - Ck^2 > 0$.

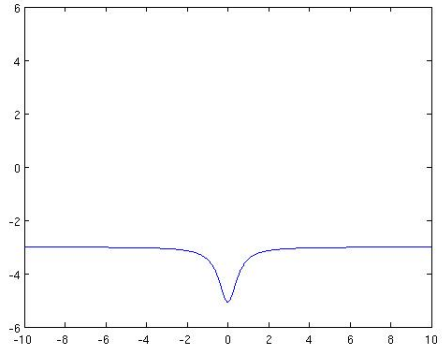

Figure 3: *x-nullcline in the case* $l - Ck^2 < 0$.

S-nullcline

$$DS_0 - DS - \frac{1}{\eta}\frac{1}{k^2+h^2}\left(\frac{lS}{k'+S}\right)x = 0.$$

The S-nullcline is represented by a second order equation in S that is

$$\frac{D\eta\left(k^2+h^2\right)S^2 - \left(DS_0\eta\left(k^2+h^2\right) - D\eta\left(k^2+h^2\right)k' - lx\right)S - DS_0\eta\left(k^2+h^2\right)k'}{\eta(k^2+h^2)(k'+S)} = 0$$

Observing that in our domain the denominator is always non zero, the solution of this equation is

$$S = \frac{T(h) \pm \sqrt{T(h)^2 + 4D^2\eta^2\left(k^2+h^2\right)^2 S_0 k'}}{2D\eta\left(k^2+h^2\right)},$$

where $T(h) = DS_0\eta\left(k^2+h^2\right) - D\eta\left(k^2+h^2\right)k' - lx$ and $x = \frac{h-h_0}{\gamma}$.
Let us observe that the term under square root is greater than $T(h)$ so that one of the two solutions is negative and not interesting for our study. The solution which represents the S-nullcline is

$$S = S(h) = \frac{T(h) + \sqrt{T(h)^2 + 4D^2\eta^2\left(k^2+h^2\right)^2 S_0 k'}}{2D\eta\left(k^2+h^2\right)}.$$

The qualitative analysis of this function is more complex then the x-nullcline. Anyway using a Matlab function (see Appendix) we can have a clear idea of the behavior of the S-nullcline. It is shown in Fig 4.

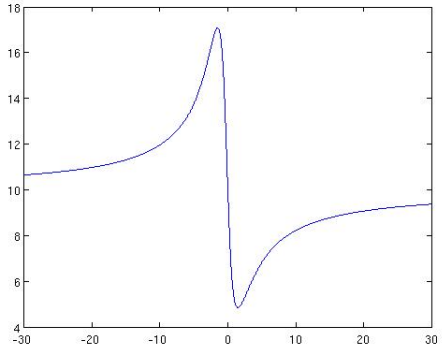

Figure 4: *S-nullcline for particular choice of the parameters. Changing the parameters values, the maximum and the minimum of the function vary but the qualitative behavior remains similar.*

2.2 Equilibria

After plotting x-nullcline and S-nullcline separately, it is important to investigate their intersections for seeking fixed points and then studying the

stability of the system. The analysis presented in this section is supported by the extensive use of the software XPP-Aut.
I have written a file *System.ode* (enclose in Appendix) in which I have defined the system (1)-(2) and chose the values of the parameters observing how the behavior of the system changes varying them. Using XPP-Aut we can plot the x- and S-nullclines and draw the vector fields that give a hint about how trajectories move around in the plane. Once having the graphical representation of the fixed points, it is possible to know precise information about the stability of the equilibria.

2.2.1 Numerical values of the parameters

The system is characterized by ten parameters. We don't know a priori information about their magnitude. After numerous simulations, we have decided to start our analysis from those values which have lead to some interesting behavior of the system, in terms of fixed points and stability, in the biological domain. In particular we initially assume $h_0 \simeq 0$ because it represents the hydrogen ion concentration at some point. From the definition of pH in the previous sections, we know that the concentration of hydrogen ions is mathematically expressed by 10 to a negative power. We also assume $\alpha \simeq 0$ because it is related to the number of cells that leave the tissue and it is very small.
We can notice that there will be always at least one intersection between the two curves because of the presence of the line $x = 0$ in the x-nullcline, this is the trivial equilibrium.

Let us start from the following parameters values:

$\alpha = 0.1$	$h_0 = 0.0001$	$m = 0.5$
$D = 10$	$k = 0.8$	$k' = 7$
$l = 100$	$\eta = 0.3$	$\gamma = 1.5$

The concentration of glucose in reservoir is initially small, $S_0 = 3$.
The resulting graph is shown in Fig 5 where we focus on the upper half plane, though the significant domain from a biological point of view is restricted to $x \geq 0$ and $S \geq 0$.
We observe three intersections: two of them are stable fixed points, labeled by a circle, the other one is unstable and labeled by a triangle. We can be sure about the stability of such points simply looking at the eigenvalues of the linearized system in the neighborhood of each point. These eigenvalues are automatically computed by XPP-Aut. They have both negative real part if correspond to a stable fixed point. On the other hand, if we are in the neighborhood of an unstable node, there is at least one eigenvalue with positive real part.

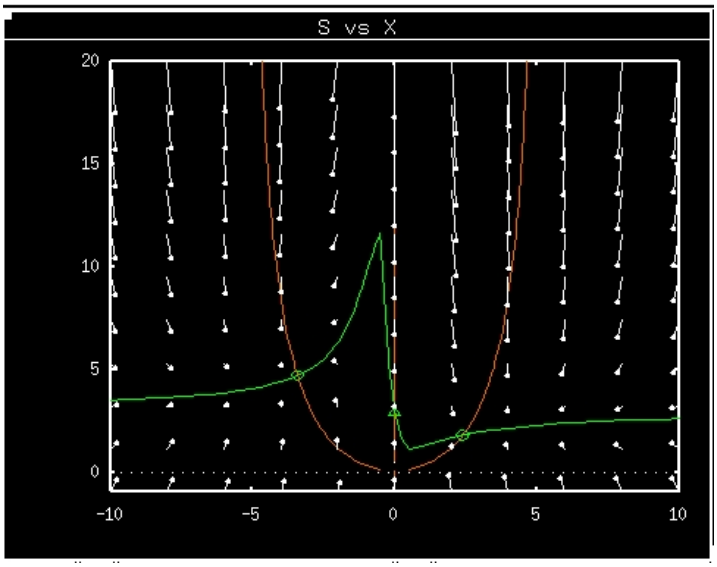

Figure 5: *The x-nullcline is plotted in red, the S-nullcline in green. They correspond to* $\alpha = 0.1$, $m = 0.5$, $\eta = 0.3$, $h_0 = 0.0001$, $\gamma = 1.5$, $D = 10$, $l = 100$, $k = 0.8$, $k' = 7$, $S_0 = 3$. *The rows represent the vector fields. We observe two stable external equilibria and one unstable in the middle.*

Another example of result is shown in Fig 6, where we have decreased the value of γ (notice that the scale on the axes is different with respect to the previous phase portrait).

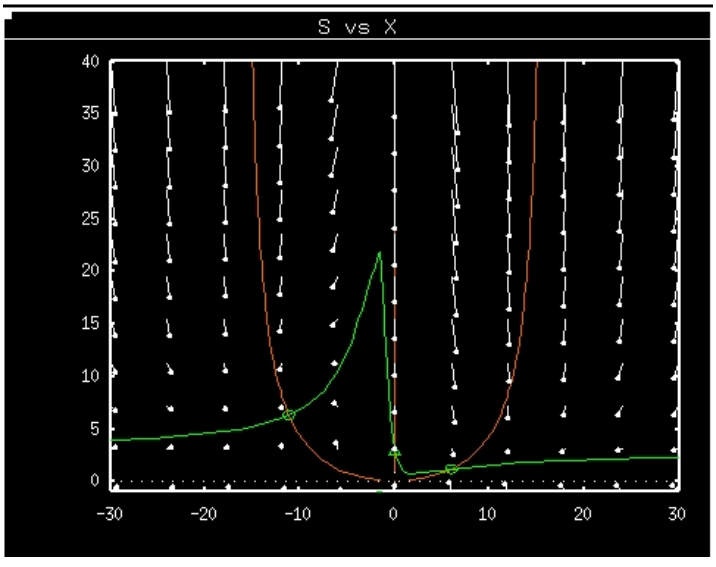

Figure 6: *The x-nullcline is plotted in red, the S-nullcline in green. They correspond to* $\alpha = 0.1$, $m = 0.5$, $\eta = 0.3$, $h_0 = 0.0001$, $\gamma = 0.5$, $D = 10$, $l = 100$, $k = 0.8$, $k' = 7$, $S_0 = 3$.

Several implementations with different values of the parameters have shown the mutual behavior of the x- and S-nullclines and have suggested the analysis of the system with respect to particular parameters: k, h_0 and S_0. The motivation of this choice is explained in the following sections where we present the mentioned analysis. We don't exclude the possibility to find new interesting results from the study of the system as function of other parameters.
Before proceeding, it is important to notice that, in the domain we are considering, there isn't the possibility to have more than three steady states. In fact it should happen if the green curve had a minimum lower then the red curve in the region $x > 0$. Several implementations with a matlab function for searching minima have shown that the minimum of the S-nullcline is "appreciably" higher then the minimum of the x-nullcline, so that the maximum number of equilibrium points we can observe is three.
Moreover, we remark that the positive state space ($x \geq 0$; $S \geq 0$) is invariant. This guarantees that positive (and biological) initial conditions give rise to positive/biological solutions. If we look at Fig 7, the curves colored in yellow and blue divide the half plane in invariant sets. In Fig 8, we can see branches of trajectories which start from different initial conditions in the positive state space and remain in the same region.

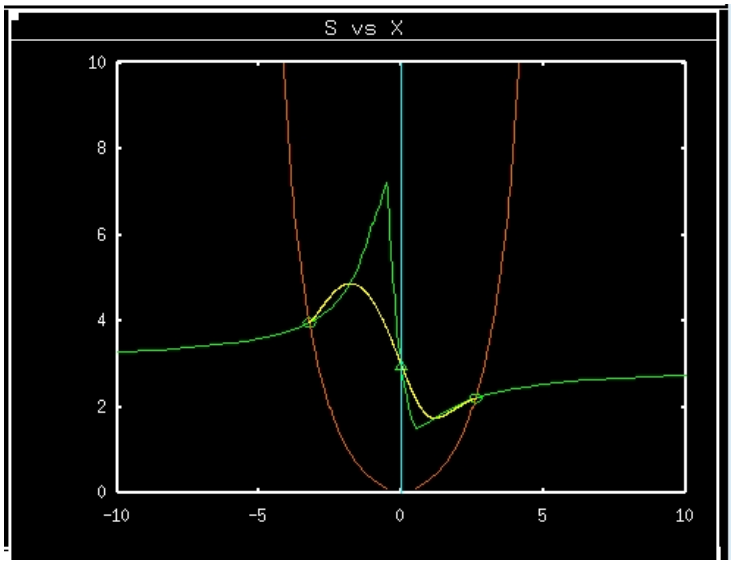

Figure 7: *The x-nullcline is plotted in red, the S-nullcline in green. The yellow and blue curves delimited the invariant sets.*

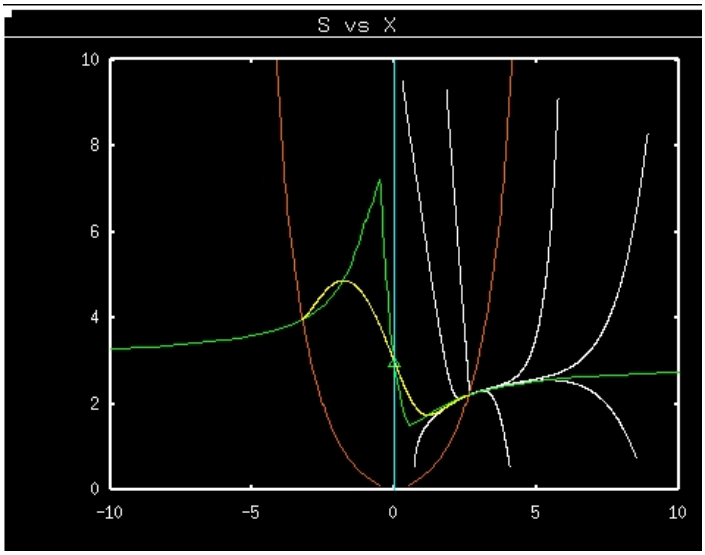

Figure 8: *Branches of trajectories starting from different initial conditions in the region $x \geq 0$, $S \geq 0$. They evolve remaining in the same space.*

3 Bifurcations and continuations

The software XPP-Aut contains a package, AUTO, very efficient for detecting bifurcations of fixed points and limit cycles. A bifurcation occurs when a small smooth change made to the parameter values (the bifurcation parameters) of a system causes a sudden "qualitative" or topological change in its behavior. Using AUTO, once known the coordinates of an equilibrium point, it is possible to plot the curve of the fixed points as function of a particular parameter. We present the analysis with respect to the parameters k, h_0, S_0.

3.1 Diagram with respect to "critical" population size

The parameter k is introduced in the system through the growth function μ that is

$$\mu(S,h) := \frac{1}{k^2+h^2}\left(\frac{lS}{k'+S}\right).$$

We can interpret k as an indicator of a "critical" population size. Let us explain in which sense. If we fix the variable S, the function μ can be seen as $1 - f(h)$, up to a constant $(1/k^2)$, where f is the Hill function $f(h) = h^n/(k^n + h^n)$ with $n = 2$. For having an idea of the behavior of μ, we can see Fig 9. As $h < k$ the function assumes its maximum value. As $h > k$, the function tends to be zero, then the growth rate is small. We recall that $h = h_0 + \gamma x$, with $h_0 \simeq 0$. In terms of cells density, we can assert

that, as $x < k/\gamma$, the growth rate is high while as $x > k/\gamma$ the growth rate is small so that we observe an inhibitory effect: x large $\Rightarrow$ function small. In this sense we say that the parameter k is related to a critical population size.

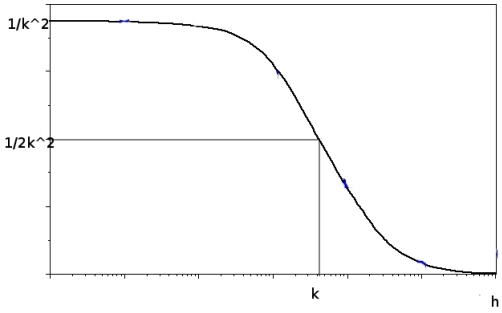

Figure 9: *Behavior of the function* $1/(k^2 + h^2)$.

Why we have decided to study the system as function of k? The motivation is based on the definition of the formula for the asymptote of the x-nullcline (8). We have observed that this line is particularly important for understanding the behavior of the system, so we have focused the attention on one of the parameters involved in such formula.
Let us observe the diagram in Fig 10 which synthesizes the behavior of the system for $0.8 \leq k \leq 6.5$. The other parameters are fixed. First of all we have a quick summary of stability from the diagram itself: thick solid lines indicate stable fixed points, thin lines are unstable fixed points. The point labeled by the number 3 is denoted, in the information window, as BP and corresponds to a bifurcation point. It has the typical features of a *pitchfork bifurcation* point, apart of a little interval discussed later. This particular point occurs at the value $k = 2.739$ and is characterized by two stable fixed points that tend to appear and disappear in symmetrical pairs. So, we can see that, as $k < 2.739$, the system performs three fixed points, two stable and one unstable.
The point labeled by 2 is denoted as LP and corresponds to $k = 2.776$. We can notice that as $2.739 \leq k \leq 2.776$, the system figures out very different situations, although the variation of the parameter is extremely small. It starts from having two stable fixed points, one stable and one unstable (see Fig 11). Then slowly increasing k, we observe three steady states (the external ones are stable and the other one is unstable) and finally, as $k = 2.776$, we have again two fixed points and both are stable. As $k > 2.776$, the system presents only one stable fixed point that corresponds to the trivial one $x = 0$, $S = S_0$.

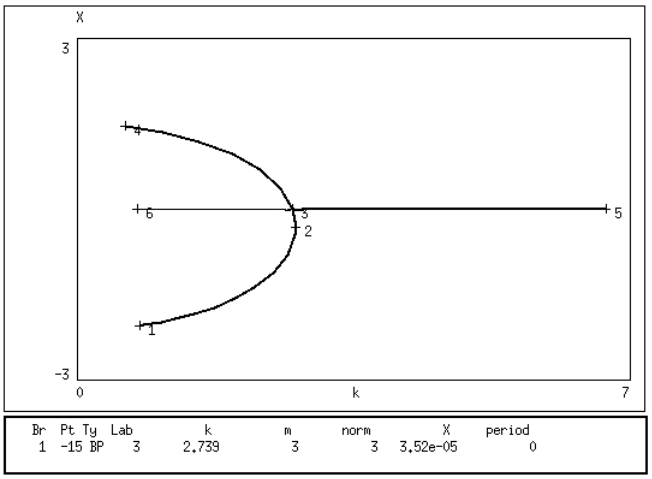

Figure 10: *Bifurcation diagram as the parameter k varies. The other parameters are fixed: $\alpha = 0.1$, $m = 0.5$, $\eta = 0.3$, $h_0 = 0.0001$, $\gamma = 1.5$, $D = 10$, $l = 100$, $k' = 7$, $S_0 = 3$. Thick solid lines indicate stable fixed points, thin are unstable fixed points.*

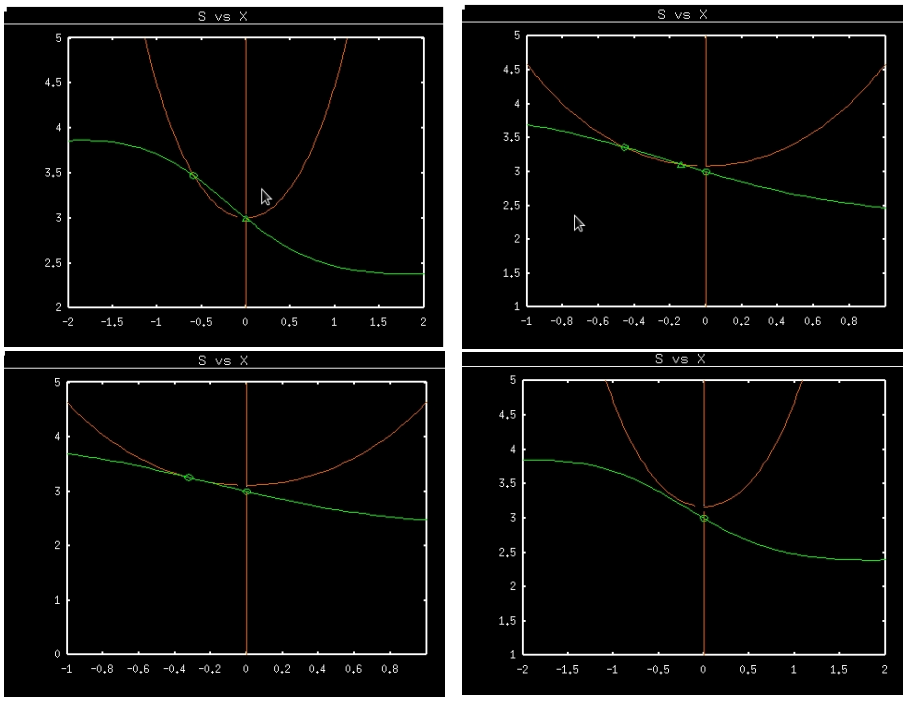

Figure 11: *The four images show how the number of the fixed points and their stability change as $2.739 \leq k \leq 2.776$ and soon after the value labeled by 2.*

The discussion above is interesting from a mathematical point of view

but needs to be restricted to the domain where our system has still a meaning from a biological point of view. So we look at the horizontal line and the upper branch. Basically the system performs only one stable point which is very close to zero if $k > 2.739$, while presents a higher stable equilibrium as $k > 2.739$. We can interpret this results saying that for big values of k, the maximum growth rate $1/k^2$ is small, then the increase of cells concentration is negligible. On the contrary, if the value of k is small, the maximum growth rate is high, then we observe an increase in the concentration of cells and we can justify the presence of a stable fixed point on the upper branch.

3.2 System behavior with respect to the tissue pH

Till now we have assumed that the parameter h_0 is extremely small. We can observe the behavior of the system when we increase the value of h_0. It is interesting because we know the particular biological meaning of h_0 that is defined as the concentration of hydrogen-ions in the tissue when there aren't cancer cells but only normal cells. Basically, the variation of h_0 corresponds to consider a different tissue, where the normal concentration of protons and then the pH are different.
Let us fixed the other parameters: $\alpha = 0.1$, $m = 0.5$, $S_0 = 3$, $\eta = 0.5$, $h_0 = 0.0001$, $\gamma = 1.5$, $D = 10$, $l = 100$, $k = 0.8$, $k1 = 7$. Plotting the phase portrait, we have the result shown in Fig 12.

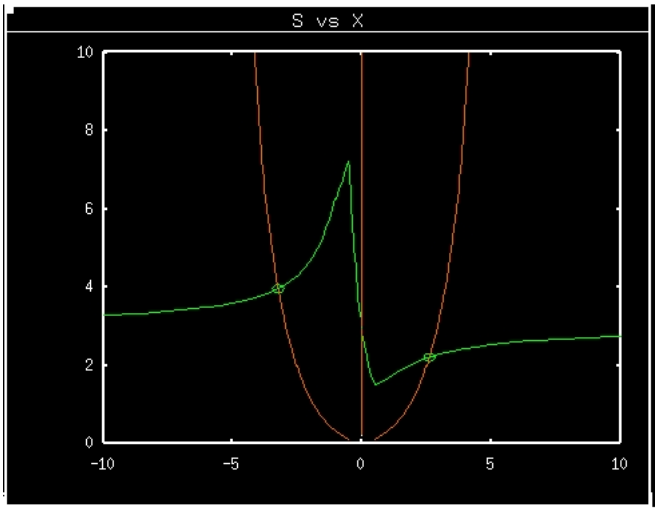

Figure 12: *The x-nullcline is plotted in red, the S-nullcline in green. The points labeled by circles are stable equilibria.*

In Fig 13, we can observe the diagram obtained starting from two different initial conditions. In the first graphics we start from the stable fixed point $x = 2.615$, $S = 2.2155$ (in the positive square). The second one is plotted setting as initial condition the stable node $x = -3.2307$, $S = 3.9692$.

It should be optimal to superimpose the two graphics but we can deduce our results easily comparing them from Fig 13, where we can notice that the line in the second diagram is always below the two lines in the first one. We find that there is a particular point labeled by 2, in the first diagram, that corresponds to $h_0 = 4.4$. Before this node, we have three fixed points, the upper one is stable, the other one is unstable ($x \simeq 0$), the last one, with negative x, is stable (this is shown in the second diagram). If we look at what happen for $h = 4.4$, we expect to obtain only two fixed points and indeed it is what we gain (Fig 14). As $h > 4.4$, the systems performs again three steady states, Fig 15.

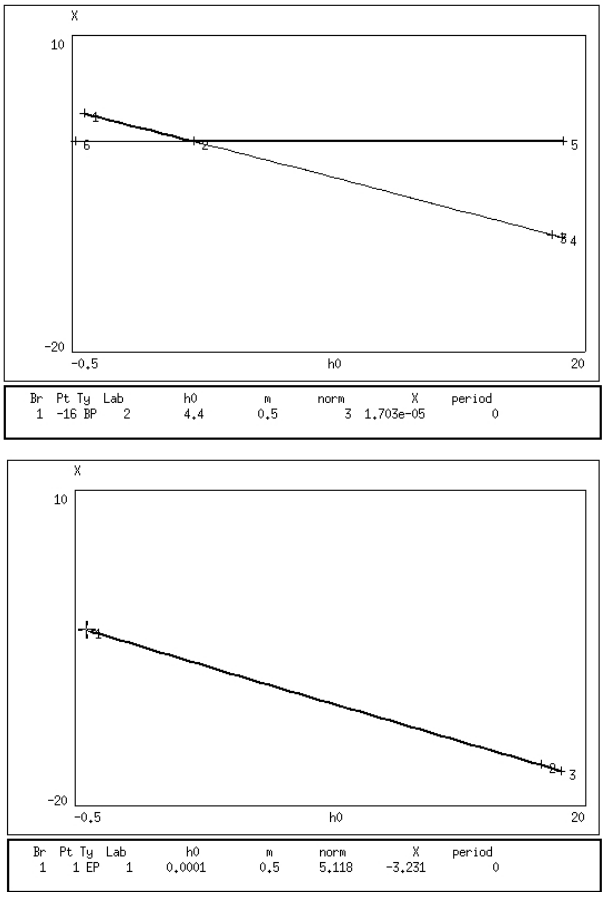

Figure 13: *Diagram representing fixed points when the parameter h_0 varies.*

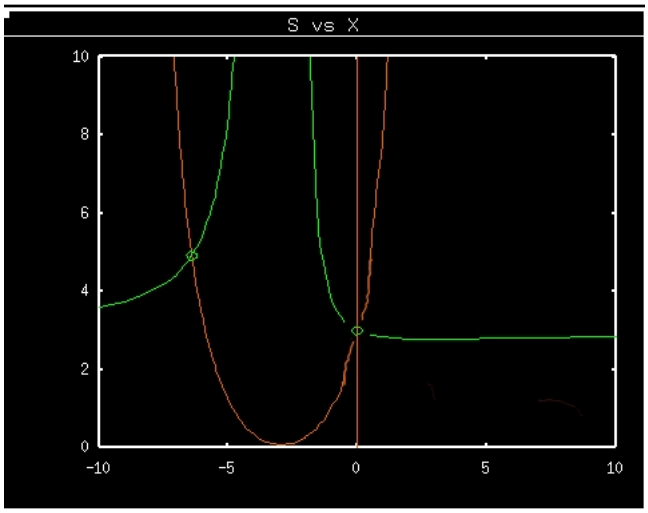

Figure 14: *x-nullcline and S-nullcline with two intersections. The graphics corresponds to* $h_0 = 4.4$.

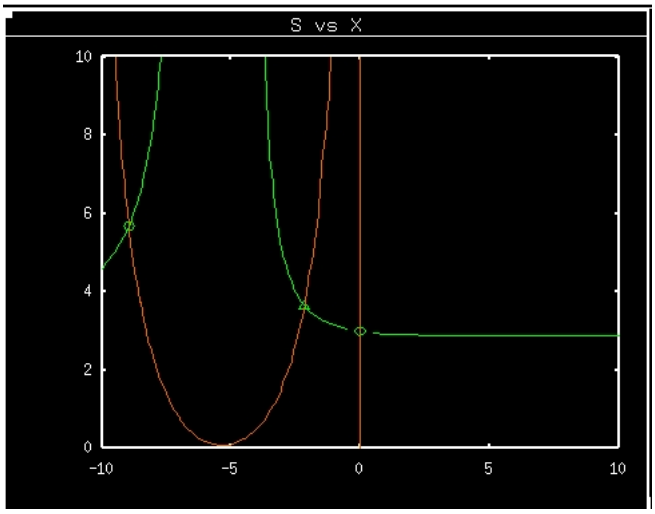

Figure 15: *x-nullcline and S-nullcline with three intersections. The graphics corresponds to* $h_0 = 8$.

Once again we remind that the system domain which is interesting from a biological point of view is $x \geq 0$ and $S \geq 0$. So let us interpret the mathematical result in such region. The study of the bifurcation diagram corresponding to h_0 is interesting because allows to find a sort of threshold, 4.4, such that, as $h_0 > 4.4$, the system performs only one stable point, the trivial equilibrium. This means that the tissue is not affect by cancer cells. Why? We recall that h_0 represents the concentration of protons in the absence of cancer cells. If such concentration is high, the pH is low,

then the tissue is characterized by high acidity that is an obstacle for cell life. Cancer cells couldn't grow in such solution because, consuming glucose, they produce protons increasing tissue acidity that becomes more and more dangerous for them.

3.3 Varying the glucose concentration in reservoir

Let us focus on the domain $x \geq 0$. We are sure to have at least one fixed point, that is the trivial equilibrium (nothing happens). Such point is stable if it is the only steady state of the system, while it is usually unstable if there is a second equilibrium point. In this case, the latter will result stable. From several simulations we have observed that, when the x- and S-nullclines intersect at a point in the state space $x > 0$ $S > 0$, this point has a bigger x-coordinate as high is the value of the glucose concentration in reservoir S_0. Basically S_0 represents an input for our system, then it is interesting to plot the curve of fixed points as S_0 varies. This variation corresponds in some way to increase the number of blood vessels coming into the tissue (angiogenesis[2]).
We start from a low value of S_0 and the other parameters fixed, then we increase the value of glucose concentration and we initially observe a consistent increase in the number of cells in the culture chamber. After a while, their growth becomes less sensitive to the initial concentration of glucose and finally any further increase of S_0 doesn't produce any increment in the number of cells. This phenomena is shown in Fig 16 where we choose some values for S_0 and we observe how the coordinates of the stable fixed point change.

[2]Angiogenesis is a physiological process involving the growth of new blood vessels from pre-existing vessels.

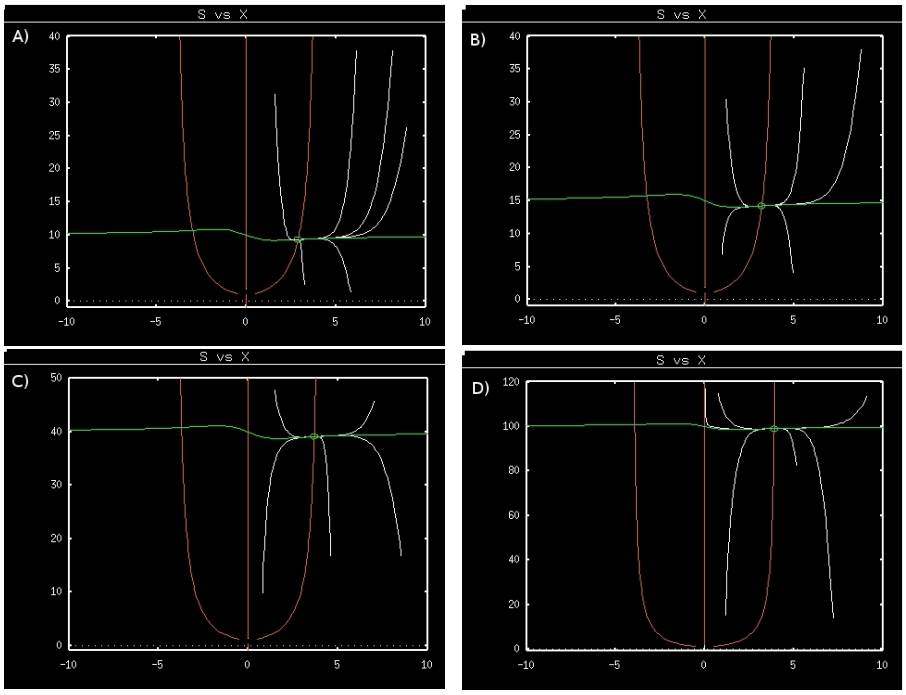

Figure 16: *A) Corresponds to* $S_0 = 10$*, the singular point is* $x = 2.8844$*,* $S = 9.327$*. B) Corresponds to* $S_0 = 15$*, the singular point is* $x = 3.1958$*,* $S = 14.254$*. C) Corresponds to* $S_0 = 40$*, the singular point is* $x = 3.6873$*,* $S = 39.14$*. D) Corresponds to* $S_0 = 100$*, the singular point is* $x = 3.9025$*,* $S = 99.089$*.*

Using AUTO, it is possible to have a graphical representation of the continuation of fixed points as the parameter S_0 changes. In this way we have the result that we expect as shown in Fig 17. The fact that the drawn curve is thick and solid implies the fixed points are stable. AUTO specially marks certain points with small crosses and numbers. As we move around the diagram, the text area beneath the diagram gives us a summary of information about the current location such as the value of the parameter, the state variable, the period, and the point type for special points. In this case there aren't special points but it is interesting to notice that after the point labeled by 8 for example, the cell density x changes very slowly as S_0 increases. In fact, as discussed in the previous sections, the red curve representing the x-nullcline is limited by a vertical asymptote, given by $h = \sqrt{(l - Ck^2)/C}$, that is

$$x = \frac{1}{\gamma}\left(-h_0 + \sqrt{\frac{l - (\alpha D + m)k^2}{\alpha D + m}}\right).$$

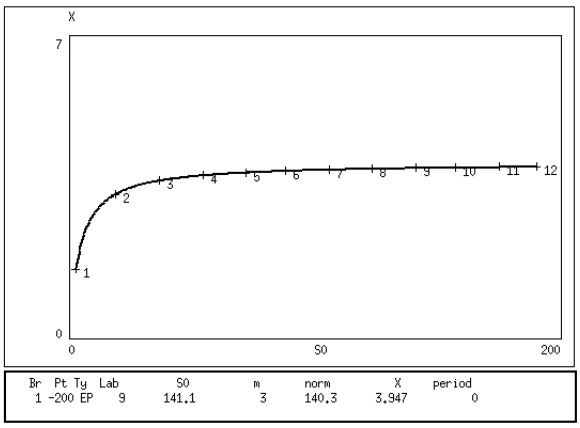

Figure 17: *The curve represents the fixed points of the system as the glucose concentration in reservoir S_0 changes and the other parameters are fixed such that the resulting asymptote is $x = 4.0603$.*

This result is very interesting because it means that, although we try to increase the concentration of nutrientin reservoir, there exist a sort of limit for the cells concentration. Starting from any initial condition, if the concentration of cells increases as the concentration of glucose in reservoir is higher and higher, we observe that at some point, it doesn't change anymore. The presence of such limit should be related in some way to the distinction between benign and malign tumor. The higher concentration of cancer cells, given by the stable equilibria, could be small in the specific case and then represent a benign tumor or big and then represent a malign tumor.

4 Conclusion

The results we have presented in this report give an idea of the behavior of a two dimensional system representing the evolution of a cancer cell population when we take into account their metabolism and the pH component. The analysis of the fixed points and their stability guarantees that the system doesn't perform any oscillation but, starting from any initial condition, always tends to a stable fixed points in the "positive" state space $x \geq 0$, $S \geq 0$. This point can coincide with the trivial equilibrium $x = 0$, $S = S_0$ which means that there aren't cancer cells growing in the tissue or with a stable fixed point lying on the red convex function limited from an asymptote. This means that, in the case we observe an increase in the concentration of cancer cells in the tissue, this concentration cannot go to infinite but will stabilize at some point. Even if we increase the nutrient solution for the cells, they wont growth anymore.
The extensive use of the software XPP-Aut and the tool AUTO have lead to a graphical approach for understanding how fixed points and stability

change with respect to particular parameters. Through this analysis, we have obtained two important results.
The first one is related to the hydrogen-ions concentration h_0 and consists on the observation that after some threshold, a big value of h_0 is an obstacle for the cancer cells. This should explain why, in the process of metastasis, cancer cells attach some organs and not others. So it could suggest a study of this process as function of the pH of the particular tissues.
The other interesting result is described in the last section, where we study the curve of fixed points as S_0 changes. The advantage of this observation is that the parameter S_0 has a precise biological meaning and its variation can be interpreted as the angiogenesis process. Starting from any initial condition of the system, if we observe an increase in the concentration of cells in the tissue, we should increase such density with a higher value of the glucose concentration in reservoir. Anyway this process could not produce an infinite increase in the number of cells because of the asymptote which represents a limit for that.
We remark that part of the mathematical analysis in the report cannot be translated into biological terms, since it involves negative values of the variables which have not biological meaning. Anyway we have chosen to mention it to better understand the whole idea.
The description of one cell population model could be a starting point for approaching the study of the more complex dynamics of two cell types in a substrate of glucose and oxygen. This model is in fact characterized by a sort of symmetry and each part can be seen as explained in this report. The presented analysis could be a hint for choosing two settings of parameters for the two populations. Then the observation of how the system evolve with respect to these parameters should be done using the graphical approach we have proposed, using the software XPP-Aut.

Appendix

File *System.ode*

```
#############################################################
#Model with one population of cell in a substrate of glucose
#############################################################
#            numerical values of the parameters
#     parameters in the first equation
par a=0.1, m=0.5
#     parameters in the second equation
par S0=3, eta=0.5
#     common parameters
par h0=0.01, gamma=1.5, D=10, l=100, k=5, k1=7
#
#            hydrogen-ion concentration
h(x)=h0+gamma*x
#
#            growth function
mu=1/(k^2+(h(x))^2)*(l*S/(k1+S))
#            differential equations
x'=-a*D*x-m*x+mu*x
S'=D*S0-D*S-1/eta*mu*x
#
#            auxiliary variable for growth function
aux growth=mu
#
@ bounds=1e25            # to prevent "out of bounds" error
@ total=1000      1      # to extend the run time
@ maxstor=100000         # to increase the maximum storage capacity
@ meth=stiff
@ xp=x, xlo=-10, xhi=10, dt=0.001
@ yp=S, ylo=0, yhi=40
#
done
```