TOPOLOGICAL APPROACHES TO BIOLOGICAL DYNAMICS

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The growth of computer power and biological data bases is revolutionizing the application of mathematics to biology. Mathematical models of biological systems are being developed at all levels of organization from the folding of protein molecules, to the development of cells and tissues, to the beating of a heart, to the evolution of ecosystems. In some cases, the mathematical model simulates the biological behavior at an extraordinary level of detail and accuracy. Yet, such models are invariably complicated and though the behavior might be similar to the biological system, the underlying principles behind the dynamics may be opaque. Of course, for some purposes it would make no difference whether or not we understand the observed behavior in the model. Thus, a sufficiently accurate model of the heart might enable us to test the effects of drugs in silico, rather than in vivo. Such a model would be useful in screening for drugs that could lead to fatal arrhythmias. There are many groups that are working towards realistic models and the potential benefit of such models will be huge.

In this series of lectures, I will focus on mathematical approaches that are useful for thinking about the underlying principles for the organization of biological dynamics. The approach is topological and qualtitative, but in some cases, can make contact with and in fact require high precision data, as in the consideration of the experimental resetting of biological oscillators. The topological approach was championed in the 1970s and 1980s by giants like Art Winfree and René Thom, whose influence and contributions are gratefully acknowledged at the outset. Winfree's masterpiece, *The Geometry of Biological Time* defines the approach and covers a huge range of topics [37].

I do not try to give a comprehensive review of all uses of topology in biology, but mostly focus on a series of results found by myself and colleagues related to fixed points and continuity in biological dynamics. The results I mention are all comparatively simple, and although some are well known, most are not. I will not attempt to be rigorous, but will try to present the material in a fashion that I hope can be understood and applied without advanced mathematics. I will not give elaborate proofs or definitions, and will assume the reader has some basic knowledge of mathematics. Whenever possible, I will give experimental or clinical biological examples to illustrate the ideas.

All of the material is based on previous publications which I cite and which should be consulted for full details and extensive references to earlier work. This material will be in lecture note style. It is hard to get notation consistent from one section to the next, so try not to be bothered if the same symbol has different meanings in different sections. Some of the material presented here is repeated from [2] and some laboratory exercises from that book will be given. The downloadable matlab code for the exercises, which I hope will work on the PCMI computers is at `http://www.cnd.mcgill.ca/ebook/`. Since the background of different people will likely be quite different, a reference for basic concepts which contains some of the examples I will give is [28]. This book also has lots of pencil and paper exercises in 1 dimensional maps and 1 and 2 dimensional differential equations for those who need practice in doing those types of problems.

1 Linear stability and the Poincaré–Hopf theorem applied to biology.

One of the fundamental techniques in the analysis of nonlinear dynamical systems is to determine the stability of a nonlinear system in the neighborhood of a fixed point. The basic technique is to find the fixed point, linearize the system in the neighborhood of the fixed point, and determine the eigenvalues of the resulting linear equation.

Consider the nonlinear ordinary differential equation,

$$\frac{dx_i}{dt} = f_i(x), \text{ for } i = 1, \ldots, n \tag{1}$$

where f_i is a nonlinear function of x. A fixed point, x^* is a point for which

$$f_i(x^*) = 0$$

for all i. The elements of the Jacobian matrix, A are determined by computing

$$a_{ij} = \left.\frac{\partial f_i}{\partial x_j}\right|_{x^*}. \tag{2}$$

The eigenvalues of of A are found by solving the equation

$$\det \mid A - \lambda I \mid = 0. \tag{3}$$

If the real parts of all the eigenvalues are different from zero the fixed point is called hyperbolic. I will assume that all fixed points are hyperbolic. If the real parts of all the eigenvalues of a fixed point are negative, then the fixed point is locally stable. This means that all initial conditions in the neighborhood of the fixed point will approach it in the limit $t \to \infty$. If one or more eigenvalues of a fixed point are positive, the fixed point is not locally stable. Some or all of the initial conditions starting in the neighborhood of the fixed point, will diverge from it as $t \to \infty$.

If the nonlinear equation is in 2 dimensions, then the eigenvalues are just the solution of the quadratic equation. If both eigenvalues are real and the same sign, then the fixed point is called a node. If the eigenvalues are real and different signs, then the fixed point is called a saddle point. Finally if the eigenvalues are complex, then the fixed point is called a focus. In the neighborhood of the fixed points, the geometry of the flow is different for each type of fixed point. This will be illustrated using some dot images that form the basis for the computer lab given at the end of this lecture.

The Poincaré–Hopf Index Theorem places restrictions on the numbers and types of fixed points in ordinary differential equations embedded on manifolds. Assume that there is a differential equation with hyperbolic fixed points. Call π_i the number of eigenvalues of the ith fixed point whose real part is positive and μ_i the number of eigenvalues of the ith fixed point whose real part is negative. For a differential equation defined on a manifold M with k fixed points, the Poincaré–Hopf Index Theorem states

$$\Sigma_{i=1}^{k}(-1)^{\mu_i} = \chi(M), \tag{4}$$

where $\chi(M)$ is the Euler Characteristic of the manifold M. For example, the surface of sphere has an Euler characteristic of 2. You probably know that the number of faces plus

the number of vertices minus the number of edges for a convex polytope is equal to 2 (think of 2 as the Euler characteristic of the sphere). In [28] I give some simple examples and discuss some informal proofs of Eq. (4) for simple manifolds. Anyone who wants the real story should look in [27].

Here are some exercises you can do to help you understand this result. Can you draw a vector field on the surface of a sphere S^2 with two stable fixed points that satisfy the theorem? What about a vector field with two unstable fixed points? The Euler characteristic of a torus is 0. Can you draw three different vector fields with no fixed points on the torus? What about a vector field with 4 fixed points?

For an oriented surface of genus k (i.e. a torus with k holes), $\chi(M) = 2 - 2k$. As an exercise, assume the Poincaré–Hopf Index is correct, and use it to derive the Euler characteristic for a surface of genus 2 by drawing a legal vector field on the surface following the example provided in [27].

What does any of this have to do with biology? Many biological models are formulated as ordinary differential equations. Since in an real system, points cannot escape to infinity, it should be possible to embed an equation with n variables in an n-dimensional ball, B^n (i.e. the set of points defined by $|x|^2 < a$, where $|x|^2 = \Sigma x_i^2$), and where all trajectories on the boundary of the ball are pointing into the ball. The result below applies to all equations of this sort. It is then possible to identify the boundary of B^n with an unstable source for which $\pi_i = n$ of the South Pole of the n-sphere, S^n (S^n is the set of points in $n+1$ dimensions defined by $|x| = a$). The Euler–Poincaré characteristic for S^n is

$$\chi(S^n) = 1 + (-1)^n. \tag{5}$$

By combining this result with Eq. (4) we are led to the following result.

Theorem 1 *For an ordinary differential defined on an attracting ball in Euclidean space, equation with k fixed points,*

$$\Sigma_{i=1}^{k}(-1)^{\pi_i} = 1. \tag{6}$$

where π_i the number of eigenvalues of the ith fixed point whose real part is positive [11, 12].

The result provides a classification of dynamical systems based on their fixed point structure. For example, the classic boring situation is a single stable, globally attracting fixed point for which $\pi_1 = 0$. More interesting, is the situation with a single unstable fixed point for which $\pi_1 = 2$, and an attractor which is a stable limit cycle oscillation. Notice, that from the perspective of this result, such a dynamic can be present in a system of any dimension, and of course we know that we can have a system with a stable limit cycles in any number of dimensions. As a final example, consider dynamics in a plane where we have dynamics with 3 fixed points, two of which are stable nodes and the third is a saddle point. This type of geometry is found often in ecological models with competitive exclusion, or in genetic models with mutual inhibition or mutual activation.

Of course, the result is incredibly limited because it does not report on the dynamics outside the neighborhood of the fixed points of the system. But I do like the notion that we can come up with a qualitative picture of the structure of the allowed flows in a huge host of biological systems without knowing anything about the system other than it does not escape to infinity.

1.1 Computer laboratory

In my lecture, I will illustrate the geometry of flows near fixed points in a plane using displays of dots [10, 15]. Assume that a differential equation is defined on a plane and there is a random distribution of initial conditions. Starting from each initial condition, the equation is integrated for a short time. Then a dot is plotted at each initial condition and at each end point. Near a fixed point, we find that the position of a dot initially (x_n, y_n) is transformed to x_{n+1}, y_{n+1} by a linear transformation in which there is a rescaling in the x-coordinate by an amount a, a rescaling of the y coordinate by an amount b, and a rotation by an angle θ, to give

$$x_{n+1} = ax_n \cos\theta - by_n \sin\theta \tag{7}$$
$$y_{n+1} = ax_n \sin\theta + by_n \cos\theta \tag{8}$$

The eigenvalues of this transformation are given by

$$\lambda_\pm = \frac{(a+b)\cos\theta \pm \sqrt{(a-b)^2 - (a+b)^2 \sin^2\theta}}{2} \tag{9}$$

Notice that these eigenvalues are for a linear transformation, rather than the linear flow in the neighborhood of the fixed point. Consequently, the interpretation of the eigenvalues is slightly different from the interpretation of the eigenvalues for a differential equation. The eigenvalues of this transformation can be related to the geometry of the transformation in the neighborhood of the fixed point at $x = y = 0$. If the eigenvalues are complex numbers, the fixed point is a focus, if the eigenvalues are real and are both inside or outside the unit circle, the fixed point is a node, if the eigenvalues are real and one is inside the unit circle, and the other is outside the unit circle, the fixed point is a saddle. If the eigenvalues are pure complex the fixed point is a center.

There is one Matlab program (downloadable from `http://www.cnd.mcgill.ca/ebook/`):

dots(a,b,thetam,numb). This program generates 400 random dots and **numb** iterates of each of these dots using the transformation above. (Use 4 iterates for better visualization but a single iterate can also suffice. To see just the random dots, with no iterates, use 0 for **numb**.) The program **dots** plots the random dots and their iterates, and calculates the eigenvalues of the transformation, which are printed underneath the figure.

To display a plot with $a = 0.95$, $b = 1.05$, and $\theta = 0.4/\pi$, type

```
dots(0.95,1.05,0.4/pi,4);
```

Exercises

1. **Parameter values to give centers, focuses, nodes and saddles.** You may wish to see what happens for particular values of the parameters. Try to find parameters that give centers, focuses, nodes, and saddles. Try increasing the angle of rotation until you can no longer perceive the geometry of the transformation.

2. **Critical parameters and visual perception.** Can you predict theoretically the critical parameters that destroy your ability to perceive the geometry? If so, this might be a good result in the field of visual perception.

3. **Bifurcation from a saddle to a focus.** Here is a problem. In general, it should be impossible to find a bifurcation from a saddle to a focus except in exceptional cases. Consider the bifurcations observed with $a = 0.95$, $b = 1.05$ as θ varies.

4. **Direct bifurcation from a saddle to a focus?** Is there a direct bifurcation from a saddle to a focus? Try to determine this (a) by looking at the pictures and (b) analytically.

   Which is simpler and which is more informative, (a) or (b)?

2 Discontinuous phase resetting

I expect that in all fields of biology in which there are oscillations, experiments have been carried out to test the effect of a stimulus delivered to the oscillator. There are several possibilities: (i) the stimulus can have no effect; (ii) the oscillation can be established following some transient but the phase of oscillation may be shifted; (iii) the original oscillation may be destroyed and replaced by a quiescent dynamics or some other type of regular or irregular dynamics. These different behaviors may be of great practical importance. For example, when we travel from one time zone to another, we hope that our internal circadian rhythm is reset. If we have an implanted anti–tachycardia pacemaker, it will deliver one or more stimuli directly to the heart when it detects an rapid abnormal ventricular rhythm with the intention of annihilating that rhythm so that the normal sinus node pacemaker can resume its pacemaking function.

We imagine that the biological system is described by some appropriate set of dynamical equations such as nonlinear ordinary or partial differential equations. It is usual to associate the oscillation with a stable limit cycle in some appropriate nonlinear theoretical model of the oscillation [37]. Recall that a *limit cycle* is a periodic solution of a differential equation that is attracting in the limit of $t \to \infty$ for all points in the neighborhood of the limit cycle. Say that the period of the oscillation is T_0. We will designate a particular event to be the fiducial event, designated as phase, $\phi = 0$. The *phase* at any subsequent time $t > 0$ is defined to be $\phi = t/T_0 \pmod 1$. The phase here is defined to lie between 0 and 1; to convert it to radians multiply it 2π.

The set of all initial conditions that attract to the limit cycle in the limit $t \to \infty$ is called the *basin of attraction* of the limit cycle. Let $x(t)$ be on a limit cycle at time t and $y(t)$ be in the basin of attraction of the limit cycle. Denote the distance between a and b by $d[a, b]$. Let the phase of x at $t = 0$ be ϕ. Then if in the limit $t \to \infty$,

$$d[x(t), y(t)] = 0,$$

the *latent* or *asymptotic* phase of $y(t)$ is also ϕ. We say that $y(t)$ is on the same *W-isochron* as $x(t)$.

The development of the concept of W-isochrons and the recognition of their significance is due to Winfree [37]. Many important mathematical results concerning W-isochrons were established by Guckenheimer [23], who considered dynamical systems in n-dimensional Euclidean space. He proved the existence of isochrons and showed that every neighborhood of every point on the frontier of the basin of attraction of a limit cycle, intersects every W-isochron. Moreover, the dimension of the frontier of the basin of attraction $\geq n - 2$.

We now consider the effects of perturbations delivered to the biological oscillation. Assume that a perturbation delivered to an oscillation at phase ϕ shifts the oscillation to the latent phase $g(\phi)$. The function $g(\phi)$ is called the *phase resetting curve.*

The *Continuity Theorem* summarizes important aspects of the effects of perturbations on limit cycle oscillations in ordinary differential equations and partial differential equations.

Theorem 2 *If a perturbation delivered at any phase of a limit cycle oscillation leaves the state point in the basin of attraction of the asymptotically stable limit cycle, then the resetting curves characterizing the effects of the stimuli, will be continuous[9].*

In general, the phase resetting curve $g(\phi)$ is a circle map $g : S^1 \to S^1$.

Circle maps can be continuous or discontinuous. Continuous circle maps can be characterized by their *(topological) degree* or *winding number.* The degree of a continuous circle

map measures the number of times, $g(\phi)$ wraps around the unit circle as ϕ goes around the circle once. For example, for oscillations associated with stable limit cycle oscillations in differential equations, for very weak perturbations in general $g(\phi) \approx \phi$, and the degree is 1. In many instances, as Winfree discusses [37], the degree of the resetting curve is 0 when the stimulation is strong. If the degree of the resetting curve is 1 for weak stimuli and 0 for strong stimuli, there must be an intermediate stimulus (or stimuli) that will perturb the system outside of the basin of attractor of the limit cycle – though whether the limit cycle is eventually reestablished depends on whether the stimulus perturbs the system to the basin of attraction of another stable attractor. Similarly, if the resetting curve is discontinuous there must be a stimulus phase or range of stimulus phases that will perturb the system outside of the basin of attraction of the limit cycle[9].

The prototypical model for a biological oscillator is the Poincaré oscillator. The equations for this are given in the computer exercises at the end of Lecture 3.

These abstract notions are directly related to experiment. The phase resetting curve can be measured experimentally. Assume once again that the marker event of an oscillation is defined as $t = 0$, $\phi = 0$. Assume that in response to a perturbation delivered at phase ϕ marker events recur at successive time $T_1(\phi), T_2(\phi), \ldots, T_n(\phi)$. Let us assume that for all j sufficiently large, the limit cycle is asymptotically approached so that $T_j(\phi) - T_{j-1}(\phi) = T_0$, where T_0 is the control cycle length.

The phase resetting curve can be determined from the values of $T_j(\phi)$ determined experimentally under the assumption that the original oscillation is reestablished eventually and that noise is negligible (of course these are mathematical assumptions, not always true in practice). It is given by

$$g(\phi) = \phi - \frac{T_j(\phi)}{T_0} \pmod 1 \tag{10}$$

where we take j sufficiently large that $g(\phi)$ converges.

There were many early reports of resetting curves that seemed to show discontinuities in phase resetting curves, see the listing in [17]. However, carrying out careful experiments requires very careful measurement of the effects of a stimulus as a phase is varied. For oscillations with long periods such as the 24 hour circadian rhythm, such experiments are virtually impossible to carry out. Further, since in all real physical and biological systems, noise is inevitable, it can be difficult to measure small discontinuities if they are buried in noise. Finally, the issue of whether or not a resetting curve is continuous or discontinuous seems mostly of mathematical interest, and the issue has not been carefully probed experimentally.

However, in the early 1980s, Michael Guevara carried out an experimental study of resetting in spontaneously beating cells from embryonic chick heart in which he measured resettting curves that were not continuous [26]. Further no stimuli led to the annihilation of the oscillation. These experiments, which I will discuss in the lecture were carried out as carefully as possible. They were disturbing since they seemed to indicate that the robust qualitative predictions of the topological approach were wrong.

A resolution for this problem has been proposed by Krogh–Madsden et al. [31]. According to our current understanding of cardiac electrophysiology, in the real biological system, there are ion channels that might open and close stochastically. It is usually assumed that there are so many of these channels that it is possible to average and that deterministic equations can be used. However, when carrying through stochastic, as opposed to deterministic models of resetting of a model oscillator, the tiny differences in current that arise from the opening and closing of a single channel, combined with the flows in the differential equation, are adequate to lead to discontinuities in simulated resetting curves, even though no stimulus

leads to an annihilation of the rhythm. From a theoretical perspective, I believe this result is important since it shows that the topological predictions for resetting do not robustly hold for resetting of all biological oscillations, contrary to what many had assumed. Further, the necessary conclusion is that the random opening or closing of a single channel can lead to grossly different dynamics (action potential or no action potential in a group of cells). I believe that this insight can help explain apparently stochastic dynamics that is sometimes observed in recording of cardiac activity in people [33].

Another example where continuity properties of resetting is practically important involves the effects of stimuli delivered directly to the heart during certain types of cardiac arrhythmias in which the heart beats abnormally rapidly (tachycardias). It is often possible to terminate a tachycardia by delivering a single pulse directly to the heart, or a sequence of several pulses directly to the heart. To consider what happens in this case, consider the simplest model of cardiac tachycardia, a pulse of activity circulating on a one dimensional ring. I assume that the ring represents an excitable media. Although real hearts are not homogeneous, also assume that the ring is homogeneous, so that if a pulse of activity was initiated at a point on the ring, it would propagate in two directions until the waves collided and annihilated at the antipodal point on the ring.

One of the basic models for excitable media is the FitzHugh–Nagumo equations,

$$\begin{aligned} \frac{\partial v}{\partial t} &= -w - v(v-0.139)(v-1) + D\frac{\partial^2 v}{\partial R^2} + I(R), \\ \frac{\partial w}{\partial t} &= 0.008(v - 2.54w) \end{aligned} \tag{11}$$

where D represents the diffusion coefficient, $I(R)$ represents injected current (the perturbation) at position R [20]. In the homogeneous equations ($D = 0$) with no injected current, there is a globally stable steady state at $v = 0, w = 0$. In response to a sufficiently large perturbation a large transient of v and w away from 0 can be elicited. This equation supports a circulating pulse on the ring, It may be surprising, but this very simple model is very close to the working model that many cardiologists have for some instances of such dangerous cardiac arrhythmias rhythms as monomorphic ventricular tachycardia and atrial flutter. The mathematical significance is that the cardiologists try to ablate a portion of the ring by using radiofrequency radiation to burn the heart tissue. If this procedure is successful, then the arrhythmia is cured. In carrying out the procedure, the cardiologist is thinking like a topologists (they do surgery on an imagined manifold).

It is interesting to think about the resetting of a circulating pulse on the ring [20, 32, 9]. By considering the effect of a pulse delivered to an idealized excitable media at different phases of the cycle, it appears that the resetting curves should be discontinuous. However, according to the continuity theorem, this could only occur if there are stimuli that lead to annihilation of the oscillation, and in the FitzHugh-Nagumo equation above, annihilation of the rhythm was found for a critical stimulus [20]. It would be great to have a general theorem for all excitable media but this is not yet possible [9]. Of course in real hearts, there can be heterogeneities which will play a role in the annihilation of reentrant rhythms [34].

2.1 Computer laboratory

The laboratory exercises for this are included with the exercises at the end of lecture 3.

3 Fixed points and the entrainment of biological oscillations

The last lecture discussed the effect of a single stimulus delivered to an oscillation. Now I consider the effects of repeated stimuli. This topic is also of practical biological significance. For example, in the heart a specialized region of the heart sets the pace and in normal circumstances, the rest of the heart is entrained or phase locked in a 1:1 rhythm with the pacemaker. However, in some circumstances, there can be rhythms in which different regions of the heart beat with different frequencies, as in 4:3 heart block where the atria have 4 contractions, for every 3 contractions of the ventricles.

The results on the resetting of oscillations discussed in Lecture 2 can be used immediately to study the effects of periodic stimulation provided the oscillation is immediately reestablished following the stimulus (i.e. the transients induced by the stimulus are short compared to the time between stimuli). In this case, we can derive an appropriate one-dimensional map to predict the effects of periodic stimulation with period t_s of a limit cycle with intrinsic period T_0. Call ϕ_i the phase of stimulus i. Then, if the phase resetting curve is $g(\phi_i)$, the effects of periodic stimulation is given by

$$\phi_{i+1} = g(\phi_i) + \tau \pmod 1 \equiv f(\phi_i, \tau), \tag{12}$$

where, $\tau = t_s/T_0$. Starting from an initial condition ϕ_0 we generate the sequence of points $\phi_1, \phi_2, \ldots, \phi_n$.

The sequence $\{\phi_i\}$ is well-defined, provided no stimulus results in a resetting to a point outside the basin of attraction of the limit cycle. If $\phi_n = \phi_0$ and $\phi_i \neq \phi_0$ for $1 \leq i < n$ where i and n are positive integers, there is a periodic cycle of period n. A periodic cycle of period n is stable if

$$\left| \frac{\partial f^n(\phi_0)}{\partial \phi} \right| = \prod_{i=0}^{n-1} \left| \left. \frac{\partial f}{\partial \phi} \right|_{\phi_i} \right| < 1. \tag{13}$$

If this product is equal to 0, the cycle is called superstable.

The *rotation number*, ρ, gives the average increase in ϕ per iteration. Calling

$$\Delta_{i+1} = g(\phi_i) + \tau - \phi_i \tag{14}$$

we have

$$\rho = \limsup_{N \to \infty} \frac{1}{N} \sum_{i=1}^{N} \Delta_i. \tag{15}$$

Stable periodic orbits are associated with *phase locking*. In $n : m$ phase locking, there is a periodic orbit of consisting of n stimuli and m cycles of the oscillator leading to a rotation number m/n. For periodically forced oscillators neither the periodicity nor the rotation number alone is adequate to characterize the dynamics. The computer exercises treat the entrainment of a simple model of biological oscillations, called the Poincaré oscillator. Periodic stimulation of the Poincaré oscillator leads to an unbelievably complicated organization of locking zones, as well as zones in which there are cascades of period-doubling bifurcations, and chaotic dynamics. Extensive studies carried out in the 1980s of the effects of periodic stimulation of embryonic chick heart cells have demonstrated that many of these exotic types of dynamical behavior can be observed in experimental systems [24, 18].

Consistent with the theme of these lectures, I would like to mention two fixed point theorems related to the phase locking of nonlinear oscillations. As discussed above, the effects of periodic stimulation of nonlinear oscillations can be described by maps. First assume that the oscillation is rapidly reestablished so that the map is a one dimensional circle map. The typical situation for sufficiently low amplitude stimuli is that the map is an invertible differentiable map of the circle [1]. An *Arnold tongue of rotation number* m/n is defined as the union of values in parameter space for which there is unique attracting $n : m$ phase locking for all initial conditions. For invertible diffeomorphisms of the circle of the form in Eq. (12), if there is $n : m$ phase locking for τ and $n' : m'$ phase locking for τ', then there exists a value $\tau < \tau^* < \tau'$, leading to $n+n' : m+m'$ phase locking. Usually, the range of values of τ associated with a given Arnold tongue covers an open interval in parameter space. For a given set of parameters the rotation number is unique. If it is rational, there is phase locking, and if it is irrational there is *quasiperiodicity*. Perhaps the classic example is the sine circle map

$$x_{n+1} = x_n + \tau + b \sin 2\pi x_n, \tag{16}$$

where b and τ are constants (think of τ as the time between periodic stimuli). The organization of phase locking zones in the (τ, b) plane (for another example see the computer exercises) $0 \leq b < 1/(2\pi)$ is typical and is called is called *classic Arnold tongue structure*. The periodic orbits lose stability via a tangent bifurcation. Arnold's classic analysis stops however at $b = 1/(2\pi)$. For greater values of b there is a remarkable structure of complex bifurcations that are incompletely understood [16].

However, based on the continuity properties of the circle map, we can assert the following theorem:

Theorem 3 *For $x_{n+1} = g(x_n)+\tau$ where g is a circle map of degree 1, with a single maximum and minimum, there are at least two values of τ at which there exist values of τ for which there exist superstable cycles for each rational rotation number [16].*

This result thus allows the Arnold tongues to be continued even when circle maps become non-monotonic.

A second fixed point result, can be found for some classes of periodically stimulate two (or higher) ordinary differential equations. For the periodically forced Poincaré oscillator (see computer exercises), we can use the Brouwer fixed point theorem to show:

Theorem 4 *For the 2-dimensional Poincaré oscillator, subjected to a periodic pulsatile (δ function) stimulus, there will be a period-1 cycle for any amplitude or frequency of the periodic forcing[19].*

This result indicates that there are major qualitative differences in the Arnold tongue structure between systems described by one dimensional maps, and systems described by two dimensional maps, since in the 2 dimensional system, there may appear to be the same type of Arnold tongue organization, but the locking is not longer the global attractor for all initial conditions.

3.1 Computer laboratory

These exercises are meant to accompany lectures 2 and 3. There is a mix of computer problems and pencil and paper problems. These exercises also lead in to the projects that I suggested.

The prototypical oscillator for studies of resetting is the Poincaré oscillator (which is also called the radial isochron clock). The Poincaré oscillator is most conveniently written in a polar coordinate system where r is the distance from the origin and ϕ is the angular coordinate. The equations are written

$$\begin{aligned} \frac{dr}{dt} &= kr(1-r), \\ \frac{d\phi}{dt} &= 2\pi, \end{aligned} \tag{17}$$

where k is a positive parameter. Starting at any value of r, except $r = 0$, there is an evolution until $r = 1$. The parameter k controls the relaxation rate. The phase, $\phi = 0$, corresponds to the upstroke of the action potential or the onset of the contraction.

We assume that perturbations are modelled by a horizontal translation to the right by a distance b. In the experimental setting, perturbations are an electrical stimulus that depolarizes the membrane. A stimulus induces (after a delay) a new action potential if it is delivered in the latter part of the cycle.

This theoretical model facilitates analytical work because of its comparatively simple analytical form. The phase resetting curve, $g(\phi)$ is given by

$$g(\phi) = \frac{1}{2\pi} \arccos \frac{\cos 2\pi\phi + b}{(1 + b^2 + 2b\cos 2\pi\phi)^{1/2}} (\text{mod } 1). \tag{18}$$

As an exercise you should derive this equation. In computations using equation (18), in evaluating the arccosine function, take $0 < \phi_i' < 0.5$ for $0 < \phi_i < 0.5$, and $0.5 < \phi_i' < 1$ for $0.5 < \phi_i < 1$.

The main theoretical insight into these formulae is that for low stimulus amplitude $|b| < 1$, there is resetting where g has topological degree (winding number 1), and for $|b| > 1$, there is resetting where g has topological degree 0, and many examples of both are discussed in Winfree [37]. Mathematical studies of the entrainment of Poincaré oscillator on which these exercises are based are [25, 29, 19]

There are two Matlab programs (downloadable from `http://www.cnd.mcgill.ca/ebook/`) you will use for this exercise:

resetmap(b) This program computes the resetting curve (new phase versus old phase) for a stimulus strength **b**. The output is a matrix with 102 columns and 2 arrays. The first array is the old phase ranging from 0 to 1. There are two points just less than and just greater than $\phi = 0.5$. These points are needed especially for the case where $b > 1$. The second array is the new phase.

poincare(phizer,b,tau,niter) This program does an iteration of the periodically stimulated Poincaré oscillator, where **phizer** is the initial phase, **b** is the stimulation strength, **tau** is the period of the stimulation, and **niter** is the number of iterations. It is valid for $0 < \tau < 1$. The output consists of two arrays:

- The first array (called **phi** in the following) is a listing of the successive phases during the periodic stimulation,
- The second array (called **beats** in the following) is a listing of the number of beats that occur between successive stimuli.

How to run the programs

- To compute the resetting curve for $b = 1.10$, type

```
[phi,phiprime]=resetmap(1.10);
```

- To plot out the resetting curve just computed, type

```
plot(phi,phiprime,'*')
```

- To simulate periodic stimulation of the Poincaré oscillator, type

```
[phi,beats]=poincare(.3,1.13,0.35,100);
```

  This will generate two time series of 100 iterates from an initial condition of $\phi = 0.3$, with $b = 1.13$, and $\tau = 0.35$. The array **phi** is the successive phases during the stimulation. The array **beats** is the number of beats between stimuli.

- To display the output as a return map, type

```
plot(phi(2:99),phi(3:100),'*')
```

  This plots out the successive phases of each stimulus as a function of the phase of the preceding stimulus. The points lie on a one-dimensional curve. The dynamics in this case are chaotic. In fact, what is observed here is very similar to what is actually observed during periodic stimulation of heart cell aggregates.

- To display the number of beats between stimuli, type

```
plot(beats,'*')
```

- The rotation number gives the ratio between the number of beats and the number of stimuli during a stimulation. This is the average number of beats per stimulius. To compute the rotation number, type

```
sum(beats)/length(beats)
```

Exercises

1. **Compute resetting curves for varying values of b.** Use the program **resetmap** to compute the resetting curves for several values of **b** in the range from 0 to 2. In particular determine the value of **b** at which the topology of the resetting curve changes.

2. **Test the periodicity of iterates of phi.** Use the program **poincare** to compute the succesive iterates of **phi** for different values of of **(b,τ)**. You will need to write a program to test for periodicity, or you could use the program **testper** from `http://www.cnd.mcgill.ca/ebook/` to determine whether or not the successive iterates of **phi** are periodic.

   (a) What do you find for different values of b and τ? What is the ratio of the number of stimuli to the number of action potentials?

(b) Find values for which there are different asymptotic behaviors depending on the initial condition.

(c) Find values that give quasiperiodic dynamics (for nonzero **b**).

(d) Can you find a period-doubling route to chaos?

3. **Dynamics over (b,τ) plane.** (Hard): Determine the dynamics over the **(b,τ)** parameter plane and draw a diagram with the results. To check that your program is functioning correctly, compute analytically the boundary of the 1:1 locking region. Does the observed locking ever depend on the initial condition?

4. **2 Dimensional Poincaré oscillator.** (Hard) For a project, I suggest you study the entrainment of the 2 dimensional Poincaré oscillator. Following the stimulus, the equations of motion take over and the analytic formula for the resetting can be computed. Consequently, if a stimulus is given when the system is at state point (r', ϕ') we have

$$\begin{aligned} r_i' &= (r_i^2 + b^2 + 2br_i \cos 2\pi\phi_i)^{1/2}, \\ \phi_i' &= \frac{1}{2\pi} \arccos \frac{r_i \cos 2\pi\phi_i + b}{r_i'}. \end{aligned} \tag{19}$$

where (r_i', ϕ_i') are the coordinates immediately after the stimulus. The effects of periodic stimulation can be represented as a 2D map.

$$\begin{aligned} r_{i+1} &= \frac{r_i'}{(1 - r_i') \exp(-k\tau) + r_i'}, \\ \phi_{i+1} &= \phi_i' + \tau (\mathrm{mod}\ 1). \end{aligned} \tag{20}$$

There are many differences between the properties of the periodically forced 2D system, and the properties of the same system in the infinite relaxation limit.

4 Unique limit cycles in model genetic networks

The material in this section has been presented in many places earlier and is based mostly on a result by Joel Pasternack and myself [14]. The current presentation is taken mostly from introductory sections of a recent paper [21].

A gene is a sequence of DNA that codes for a sequence of amino acids that constitute a protein. Even though all cells have the DNA code to make a vast array of different proteins, a variety of regulatory mechanisms, still not completely understood, determine which proteins will be synthesized in each cell. One mode of gene regulation is through a class of proteins called transcription factors. Transcription factors bind to the DNA, turning "on" or "off" the synthesis of specific mRNA molecules. These are in turn translated into proteins, some of which may be transcription factors and thus may influence mRNA synthesis. There are many different mathematical models of genetic networks. I discuss a highly idealized model that reduces to a piecewise linear equation. Consequently, analytic computation is possible.

Let x_i represent the concentration of chemical species i in a cell. The time rate of change of x_i is

$$\frac{dx_i}{dt} = h_i(\mathbf{x}) - \frac{x_i}{\tau_i}, \quad i = 1, \ldots, N, \tag{21}$$

where there are N chemical species, $\mathbf{x}$ is a vector giving their concentrations, h_i is a function giving the control of the synthesis of the i^{th} chemical species by the others, and τ_i is a decay constant.

In what follows, I adopt a highly simplified but nonlinear form for the functions h_i. To each continuous variable $x_i(t)$, we associate a discrete variable $X_i(t)$,

$$X_i(t) = 0 \text{ if } x_i(t) < \theta_i; \text{ otherwise } X_i(t) = 1 \ , \tag{22}$$

where θ_i is an arbitrary real-valued threshold. The key restriction we place on Eq. (21) is that the synthesis rates depend only on discrete states of chemical species.

$$\frac{dx_i}{dt} = f_i(\mathbf{X}(t)) - \frac{x_i}{\tau_i}, \qquad i = 1, \ldots, N, \tag{23}$$

where $\mathbf{X}(t)$ is the Boolean vector of discrete states of the chemical species at time t. In the biochemical context, in which the x_i are meant to represent concentrations, we assume all x_i are nonnegative and all τ_i and θ_i are positive. In general, however, we need not restrict the ranges of the x_i or θ_i, and we refer to the x_i as elements in an interacting network.

Equation (23) is piecewise linear in $\mathbf{x}$, with the pieces defined by the threshold hyperplanes $x_i = \theta_i$, and hence is easily integrated. As long as variables cross threshold hyperplanes transversally, there will be a finite or countably-infinite sequence of switching times, $\{t_1, t_2, t_3, \ldots\}$, at which some element of the network crosses its threshold. We can obtain the solution of Eq. (23) for each variable x_i for $t_j < t < t_{j+1}$:

$$x_i(t) = x_i(t_j)\, e^{-(t-t_j)/\tau_i} + \tau_i f_i(\mathbf{X}(t)) \left(1 - e^{-(t-t_j)/\tau_i}\right). \tag{24}$$

Thus, for any point in the orthant defined by the Boolean vector $\mathbf{X}$, element i asymptotically approaches $\tau_i f_i(\mathbf{X})$. We call the vector of these values the *focal point* for the orthant. If the focal point is within the orthant, then it is an asymptotically stable fixed point. If it is within another orthant, then some variable will cross its threshold, and the trajectory

continues in the adjacent orthant. We assume that for all $\mathbf{X}$, $\tau_i f_i(\mathbf{X}) \neq \theta_i$, so that no focal points lie on threshold hyperplanes.

The flow is therefore piecewise focused, with the focal points depending in general on the current orthant. As a consequence of variables crossing thresholds, the logical state changes and so might the focal point that the solution approaches. It is possible to piece together the trajectories and determine the dynamics for future times once the logical structure of the network and the parameters are known.

The logical structure of the differential equation can be represented as a directed graph on an N-dimensional hypercube (N-cube). The N-cube has 2^N vertices, each corresponding to a logical state, $\mathbf{X}$, of the network and hence to a region of state space. A directed edge between two adjacent vertices indicates a change in the logical state of the network that can be observed from some initial condition. Thus, edges in this representation represent flows across the boundaries of adjacent orthants of state space. The orientations of the directed edges in the graph can be determined by checking, for each $\mathbf{X}$ and i, whether $\tau_i f_i(\mathbf{X})$ is greater than or less than θ_i.

In certain special cases, knowledge about the dynamics in the differential equation follows immediately from the N-cube representation. A *stable vertex* on the N-cube is a vertex with no outgoing edges. A cycle is a directed path on the N-cube that starts and ends on the same vertex. A *cyclic attractor* is a cycle on the N-cube in which each vertex on the cycle has $N-1$ edges directed towards it, and one edge directed away from it. If an N-cube has no cycles, then in the limit $t \to \infty$, solutions of Eq. (23) approach a stable fixed point corresponding to one of the stable vertices in the N-cube. Which vertex is reached depends on the initial condition when there is more than one stable fixed point.

A cycle on the N-cube is a necessary condition for a cycle in Eq. (23). For situations in which there are cycles, it is useful to compute the Poincaré map describing the return to a hyperplane between two adjacent orthants.

If all the decay constants τ_i are equal, the maps that take the flows from one orthant boundary to the next have a simple form called a linear fractional map

$$M(\mathbf{x}) = \frac{A\mathbf{x}}{1 + \langle \phi, \mathbf{x} \rangle}\,, \tag{25}$$

where the thresholds are translated to zero, $\mathbf{x} \in \mathbf{R}^N$ is a point on the initial orthant boundary (in an N-dimensional network), A is an $N \times N$ matrix, $\phi \in \mathbf{R}^N$, and $\langle \phi, \mathbf{x} \rangle$ represents a vector dot product between ϕ and $\mathbf{x}$. This form for Eq. (25) follows directly from the solution of Eq. (24), where A and ϕ depend on the focal point coordinates f_i of the flow for the orthants being traversed. The composition of two linear fractional maps of the same dimension is once again a linear fractional map. As a consequence of this property, if there is a cycle we can analytically (usually with the assistance of a computer) compute the return map for a given cycle of orthants starting on a particular orthant boundary crossing. The return map is often called the Poincaré map, which can be represented as in Eq. (25), but where A is an $(N-1) \times (N-1)$ matrix, and ϕ and $\mathbf{x}$ are $(N-1)$ vectors [14].

If the directed graph on the N-cube displays a cyclic attractor, by a change of coordinates we can represent A as a positive matrix and ϕ as a positive vector. The limiting dynamics of Eq. (25) under iteration can be analyzed by application of the Perron–Frobenius theorem [14].

Theorem 5 *For a differential equation of the form Eq. (23) for which there is a cyclic attractor in N-cube representation, then one of the following two situations holds:*

1. *There is a unique stable limit cycle in phase space which passes through the orthants in the same sequence and order as the cyclic attractor in the state transition diagram. This is attracting for all regions of phase space associated by the cyclic attractor.*

2. *The trajectories in the regions of phase space associated with the cyclic attractor asymptotically spiral towards the point defined by the intersection of the threshold hyperplanes.*

The first case applies if the leading eigenvalue of the matrix A is greater than 1. Otherwise the second case applies. This result enables us to identify large numbers of stable limit cycle oscillations in high dimensional dynamical systems.

An interesting biological example is provided by the design of a genetic circuit in *E. coli* in which there are several genes in a feedback loop where each gene controls the synthesis of the next in the loop by an inhibitory interaction.

As a simplified model, we assume that there are N genes governed by the differential equations [12]

$$\frac{dx_i}{dt} = \frac{{\theta_i}^n}{{\theta_i}^n + x_{i-1}^n} - x_i \quad i = 1, \ldots, N. \tag{26}$$

where θ_i is a threshold constant, which we will assume is 0.5, and we identify $x_0 = x_N$.

When $N = 2$, the equations represent mutual inhibition, and provided $n > 2$, there are two stable fixed points and an unstable saddle point illustrating one of the generic geometries for phase planes (see Lecture 1). Gardner and colleagues constructed a bacterium with this circuit and it behaved like a toggle switch [8].

When $N = 3$, this equation displays a stable limit cycle oscillation provided $n > 4$. Elowitz and Leibler constructed a bacterium with this circuit and it displayed periodic oscillations [6]. They called this network the "repressilator".

4.1 Computer laboratory

I do not have computer code for this topic, but by now you may be pretty good in generating your own. Write a program to numerically integrate Eq. (26) for $N = 2$ and $N = 3$, and investigate the behaviors for several values of n until you have convinced yourself that you have found all the qualitatively different behaviors that are possible. A simple Euler method should be adequate. Compare the qualitatively different dynamics with the classes of different dynamics discussed in Lecture 1.

For $N = 2$, analytically compute the value for one fixed point and determine the stability of this as a function of n. For $N = 3$, analytically compute the fixed point, and determine the value of n where a Hopf bifurcation occurs leading to instability. Now numerically compute the period of the oscillation as a function of n. The theoretical value for the period in the limit $n \to \infty$ is $-6 \ln(\frac{-1+\sqrt{5}}{2}) = 2.88727\ldots$ What do you find?

As an analytic exercise derive the period of the repressilator analytically in the $n \to \infty$ limit when $\theta_i = 0.5$. You need to use the analytic solution of the equations, Eq. (24). Shift the fixed point to 0, and try to use symmetry to make the problem simpler. The numerical integration of the three dimensional system may help you to see how to do this. If you do this correctly, you will find that period involves solution of a quadratic equation.

If you enjoy doing these problems, you could write a computer program to integrate the piecewise linear equation, and study randomly generated networks searching for ones that give chaotic dynamics (this could not be done in an afternoon however, and would be a longer project!). See [5] for a recent reference.

5 Fixed points in phase maps with applications to development and spiral waves on spheres

In many situations it is convenient to label each point in some topological space with a point of the unit circle. Such a representation is called a phase map. An example is the longitudinal coordinates on the globe. For phase maps, there may be points where the phase is not defined. These are called phase singularities. An early paper that inspired me in this area was the "clockface" model for developmental biology that assumed that there was a polar coordinate system associated with certain developmental processes, and that regeneration could often be thought by imposing continuity of phase values in a two-dimensional system [7]. I presented an analysis of this work early on [13], but the topological aspects have not been well developed and are still of some interest [4], see also the Cruz-White's recent Ph.D. thesis [3].

In this lecture, I will only talk about phase maps on surfaces, but phase maps in 3 dimensions are also of great importance, for example, see the work by Strogatz and Winfree e.g. see [36] and subsequent papers as discussed in [37].

The mathematical description of spiral waves is based on the notion of phase which in turn allows one to characterize spiral waves by an index. From this description, a number of topological results placing restrictions on spiral wave dynamics can be derived [13, 36, 35, 37, 3]. The current discussion is based on work originally presented in [13], but follows the presentation in [4] which should be consulted for more details and references.

With the exception of a finite number of singular points, with each point in an orientable and compact two-dimensional differentiable manifold M we identify a unique phase lying on the unit circle, $\Phi \in S^1$. The resulting phase map or phase field is assumed to be continuously differentiable, except at the singular points. The manifold can be triangulated (subdividing it into a set of polygons), where none of the edges or vertices of the polygons pass through a singularity. The index, I (sometimes also called the topological charge or winding number) of a curve C bounding a polygon is found by computing the line integral

$$2\pi I = \oint_C \nabla\Phi \cdot d\mathbf{l}, \tag{27}$$

where the polygon is always traversed in a clockwise orientation. By continuity of $\nabla\Phi$, I must be an integer. The index of a singular point is uniquely defined as the index of any curve C provided that C encircles the point but no other singular points. The index of a curve that does not enclose any singular points is obviously zero.

If the manifold M has no boundaries, each edge of the triangulation is an edge of two polygons. Since the line integral adds up the change in phase along the various edges of the polygon, the sum of the indices of the singular points for a phase field in M can be computed.

Theorem 6 *For a phase map on an oriented two dimensional manifold with with a finite number of isolated singularities*

$$\Sigma_{i=1}^{k} I_i = 0, \tag{28}$$

where the sum is over all the singular points.

This follows since the contribution of the change in phase of each edge to the total integral is counted twice, but since the edge is traversed in opposite directions each time, the net

contribution of each edge is zero. However, unlike the more familiar Poincaré–Hopf index theorem that I discussed in Lecture 1, the sum of the indices of the singular points does not depend on the genus of the surface.

This index theorem for manifolds without boundaries can be extended to manifolds with boundaries. In the following, we will consider the case of structures that arise from puncturing orientable and compact two-dimensional differentiable manifolds. The index of a hole can be uniquely defined as the index of a curve C provided that C encircles the hole but no other holes or singular points and C is positively oriented with respect to the domain which contains the hole and is bounded by C. Applying this definition and taking the summation in Eq. (28) over the singularities and the hole, or the holes if there is more than one hole, the index theorem can be proven by the same line of arguments as for the case of manifolds without boundaries.

A sphere with a hole is topologically equivalent to a disk, and, indeed, the results for disks and for spheres with holes are consistent: For the disk D^2, bounded by a curve C,

$$\Sigma_{i=1}^{k} I = \oint_C \nabla\Phi \cdot d\mathbf{l}, \tag{29}$$

so that the sum of the indices of the singular points in the disk is equal to the index of the curve C bounding the disk. If there is a single singular point on the disk, with an index of $+1$, the index of the curve bounding the disk will also be $+1$. Imagine now the boundary of the disk to be brought together (like a draw-string bag) so that the boundary of the disk now defines a hole in the sphere. In this geometry the curve C will be traversed in an opposite orientation (the hole is now inside C) from the direction it was traversed when it was the boundary of the disk. Now if there is a singular point with an index of $+1$ on the sphere, the index of the hole is -1, so that the sum of the indices is again zero.

Since it is necessary to conserve the sum of the indices, singularities of index ± 1 usually arise and are destroyed in pairs of opposite sign [36]. An exception occurs when singularities are destroyed by collision into a boundary, so that the index of the singular point and the index of the boundary both change simultaneously. Another exception occurs if there are singularities with index different from one. In such cases interactions between different singularities can lead to destruction or creation of odd numbers of singularities [38].

There are many different applications of these results in biology and chemistry including regeneration [7], chemical and biological waves on spherical surfaces [4]. Perhaps the most exciting application is the analysis of fibrillation, a fatal cardiac arrhythmia that many believe is associated with rotating reentrant waves on the heart. If this is the case, then stimuli that terminate fibrillation must be capable of eliminating all phase singularities from the heart [30, 22]. The strong shocks needed to do this are quite different from the small shocks that can be capable of terminating some types of tachycardia which were discussed in lecture 3.

I do not have any computer exercises for this topic, but perhaps some of the earlier exercises still have material that could be completed.

Leon Glass, Montreal, June 27, 2005

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