CANCER CELLULAR DYNAMICS

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Version 2

We extend our examination of cancer spatio-temporal dynamics and add a Markov chain model for malignant cellular transitions. From this we are able to develop a simple and closed form solvable model for the average number of malignant cells of a specific type at a specific location at a specific time. We examine the diagnostic, prognostic and therapeutic implications of this methodology.

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1 INTRODUCTION

We have previously introduced a cancer cell propagation model in earlier discussions which others have also considered. However our model is for a single cell type which proliferates diffuses and flows. The rates of each are dependent on where the cell is. Namely the rates of proliferation, diffusion, and flow depend on the external environmental factors. Thus we often find that proliferation may be enhanced when a cancer cell has metastasized to the liver or brain. The cancer cell may just flow and not proliferate in the blood stream. And the cancer cell may just diffuse at it moves through the vascular wall. The environment is a key determinant.

We now add other elements, namely the probability that a cell can mutate and that as it mutates the factors related to the propagation model may also change. We know that cancer cells do not just have one mutation, but a process of such mutations. The cell may start with a specific change, such as loss of E cadherin for a melanocyte which then allows the cell to move from the basal layer. This may result in a melanoma in situ.

Then we get a mutation in BRAF which allows for proliferation of the movable melanocytes and then loss of p53 for example. Thus there may be a progression of genetic or epigenetic changes in the cell. We now develop a Markov model for this progression, and then we identify collections of cells with the same mutations and apply the same proliferation, flow, and diffusion to each. We calculate a similar diffusion equation now for the average number of malignant cells by region and by type.

1.1 ADDED COMPLEXITY

The previous model described a single mutation. This expands the model by addressing multiple mutations in a Markov manner. That is we demonstrate:

1. The standard diffusion-flow-proliferation model applies on a per-region and per cell type basis. This means that the constants we have developed previously will depend on the specific cell type as well, namely how many mutations have occurred.

2. That we know there are multiple mutations in cancer cells. Some may have a few and are indolent and others may have many and be aggressive. We develop a Markov model for such cell progression.

3. We combine the three element spatio-temporal model with the Markov cell mutation model and this allows us to determine the average number of cells of a specific type in any part of the body at any point in time.

4. We then discuss how one may use this model for prognostic and therapeutic purposes.

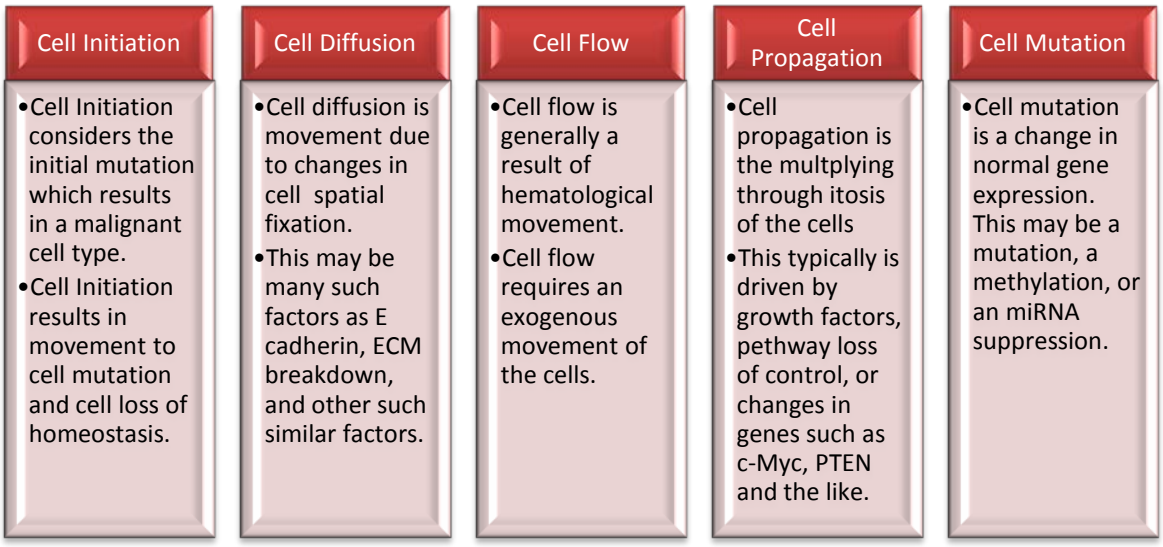
The main observation in this brief section is that the average number of malignant cells of a specific mutation state can be determined by the following equation:

$$\frac{\partial \overline{n(x,t)}}{\partial t} = \tilde{L}\overline{n(x,t)} + \Lambda \overline{n(x,t)}$$

In this equation the n is an NX1 vector of average numbers in spatio-temporal dependent values of each of N possible mutations and the L value is the spatio-temporal dependent operator matrix and Λ is a matrix describing the Markov transition probabilities between mutations.

It should be clear that we can measure all of the constants involved and thus determine the result. As a counter-distinction we can measure the n values and mutation states and determine the constants.

The expanded model considers the issue diagrammed below:



The next issue is the ability to determine what the factors are in the specific model, namely the values of the constants, and secondly the validation of the model itself.

1.2 KEY ISSUES

Thus there are two dimensions of key issues here:

1. Model Identification and Validation: In previous work we referred to this as the Observability problem. Namely if we have a model and we can identify the required parameters, then can this model be used to determine the end state which will be attained. This is the prognostic problem.

2. Model Utilization: As with the previous cases, if we have this model, and we have identified the constants, can we determine actions which may be taken to control the end state of the system? This is the Controllability problem. It states that perhaps having such a model we can determine methods and means to drive the system, in this case the average number of malignant

cells of genotype say G, to a new end state, one where we have reduced the number of bad cells to a de minimis level. This is the therapeutic problem.

There also is a third element:

3. Identification: In both of the two previous issues we assumed that there existed a method by which we could determine the constants of diffusion et al and furthermore that we could ascertain the list of possible mutations, and also their Markov transition probabilities. This may be accomplished in two ways. First, we can accomplish this by in vitro studies. Second, we can achieve this by using the model itself in a classic system identification model with in vivo analyses.

Thus the analysis contained herein is an initiation of what appears to be an innovative way to look at cancer. There have been many studies in more specific and segmented areas but there has not to my knowledge been a study that has examined cancer in such a broad and overarching manner. In essence we have included all of the variables that one may ask for.

1.3 UNDERSTANDING METASTASIS

This model is one which attempts to understand metastasis from two dimensions. First, we have examined the movement of malignant cells around the body. The movement and proliferation is driven by the cell dynamics such as the mitotic cycle and its control and the loss of cell specificity and spatial stability.

Metastasis is a somewhat unique characteristic of cancer. Viral diseases, such as HPV and similar viruses, which cause warts, are highly localized. They cause proliferation but localization is maintained.

Finally, recall that with metastasis we have the following typical sites[1]:

Cancer type	Main sites of metastasis*
Bladder	Bone, liver, lung
Breast	Bone, brain, liver, lung
Colorectal	Liver, lung, peritoneum
Kidney	Adrenal gland, bone, brain, liver, lung
Lung	Adrenal gland, bone, brain, liver, other lung
Melanoma	Bone, brain, liver, lung, skin/muscle
Ovary	Liver, lung, peritoneum
Pancreas	Liver, lung, peritoneum
Prostate	Adrenal gland, bone, liver, lung
Stomach	Liver, lung, peritoneum
Thyroid	Bone, liver, lung

[1] http://www.cancer.gov/cancertopics/factsheet/Sites-Types/metastatic?utm_source=feedburner&utm_medium=feed&utm_campaign=Feed%3A+ncifactsheets+%28NCI+Fact+Sheets%29

Uterus	Bone, liver, lung, peritoneum, vagina

Thus for each of the above sites we have a local cellular and extracellular environment which supports the metastatic behavior that we often see in such cells.

Finally we discuss some of the issue of how do we ascertain the constants in each of the models and this includes the Markov transition probabilities. We examine several approaches, invasive and non-invasive ones. We believe that molecular functional imaging, MFI, provides an attractive approach to ascertaining these constants.

2 SINGLE CELLULAR TYPE

We have previously developed a simple model for the change in the number of cells of a specific type at a specific place and time as follows.

2.1 THE MODEL

We have demonstrated earlier that for a specific type of single mutated cell that the number of such malignant cells at a specific time t and place x are determined by n(x,t) and that this can be described by the following equation.

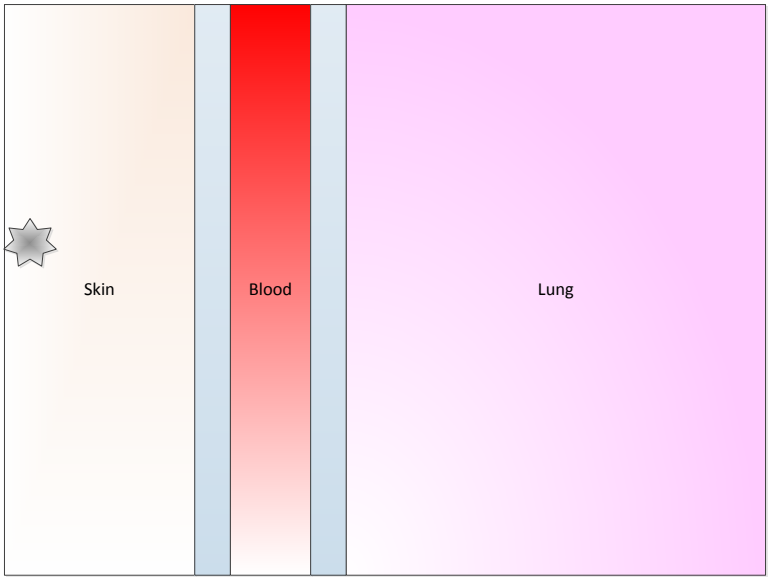
$$\frac{\partial n(x,t)}{\partial t} = a\frac{\partial^2 n(x,t)}{\partial x^2} + b\frac{\partial n(x,t)}{\partial x} + cn(x,t)$$

This depicts; diffusion, flow, and growth. The coefficients are cell type dependent and may also be spatially and temporally dependent also. We need not worry about that at this time.

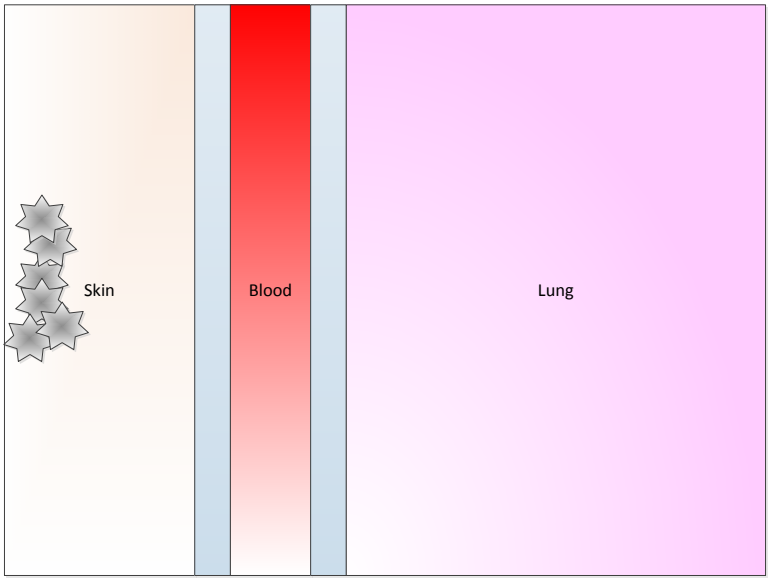
2.2 AN EXAMPLE

To better understand we depict the progression of melanoma below with a simple graphical example. We start with a simple benign cell, assume a single malignant change and then follow the proliferation and movement of the cell. This graphic makes many simplified assumptions which we shall release shortly..

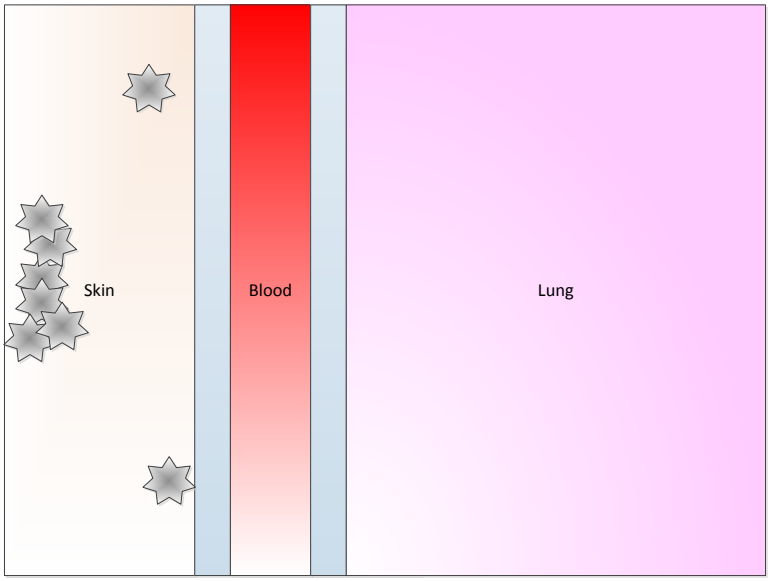
Step 1: Benign State, here we have five segments; skin, two tissue-blood barriers, blood, and lung. We begin by showing a single melanocyte. We assume the melanocyte is affixed to the basal layer with E cadherin functioning properly.



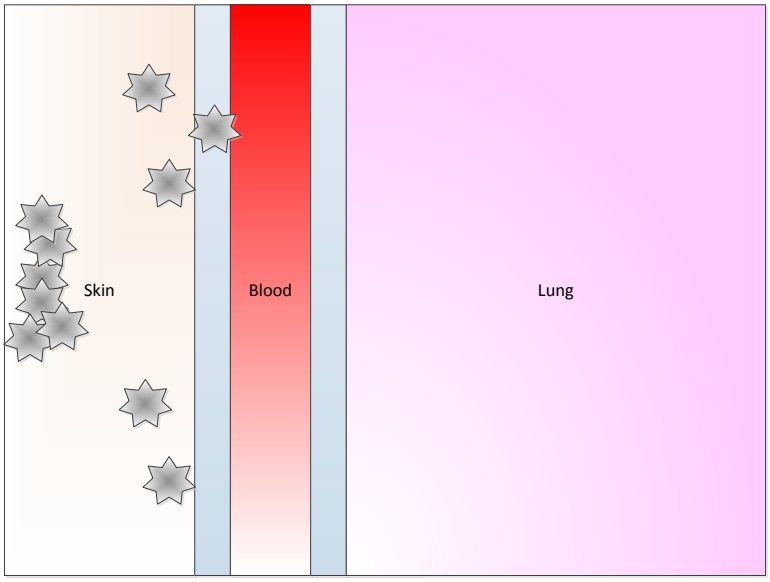
Step 2: We have the beginning of a cancer due to some mutation of the basal or luminal cells. The cancer initially proliferates and then it diffuses. In the figure below we show that it is still localized.



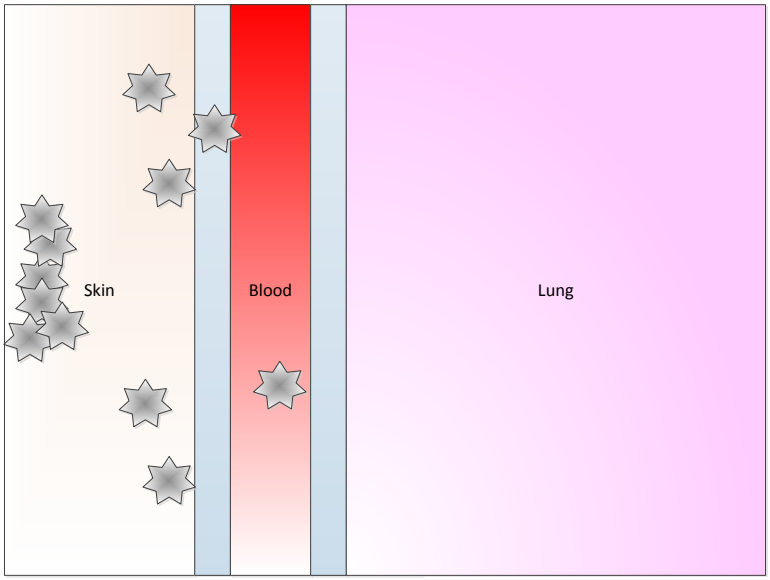
Step 3: Diffusions begins and starts to send the cancer cells towards the blood barrier.



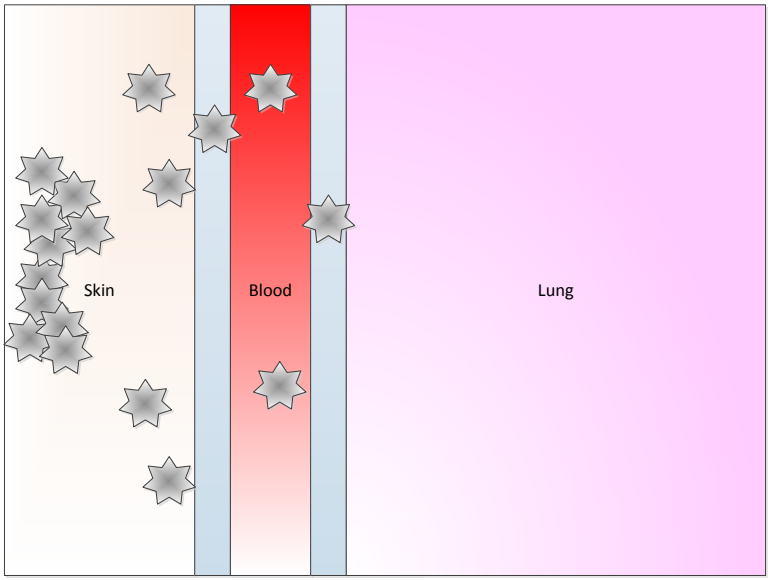
Step 4: The blood barrier is crossed, and we assume by diffusion. Across this barrier there is no proliferation or flow, just diffusion.



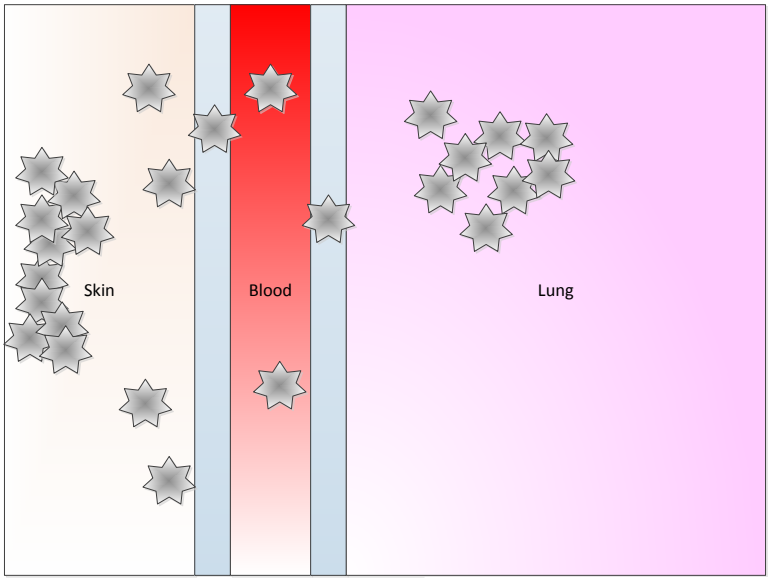
Step 5: The blood barrier is crossed and the cell is now in the blood stream. Here we have flow but no diffusion and no proliferation.



Step 6: The blood barrier is crossed again as discussed above.



Step 7: Metastasis is complete by having the new malignant cells in the lung and proliferation and diffusion predominate.



The above steps are common is almost all cancers. The assumptions here are:

1. The same malignant cell moves across the body.

2. Each separate area, in this case five, has constant diffusion, flow and proliferation constants.

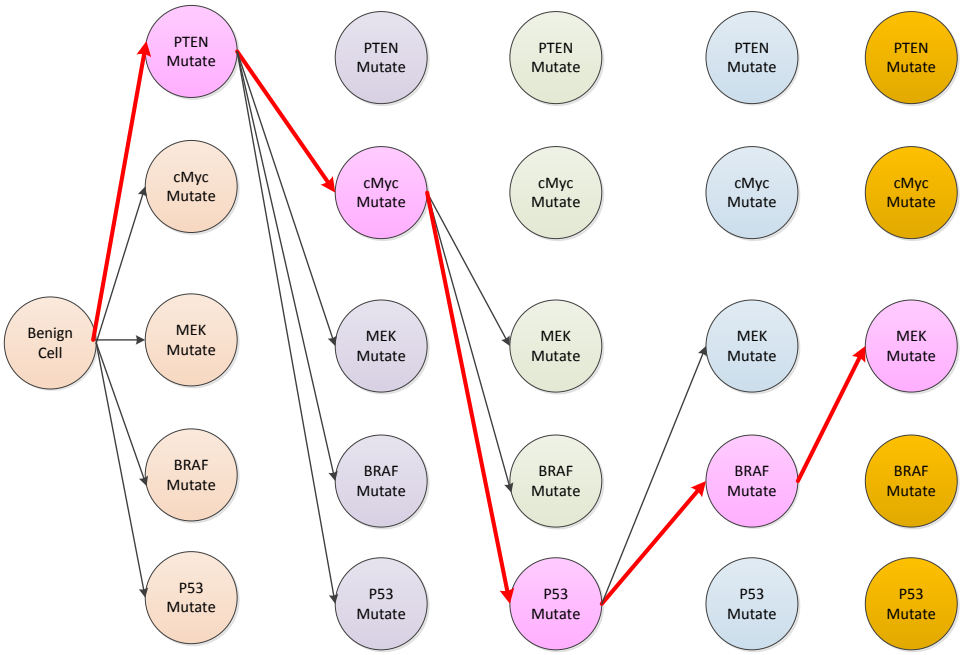
3. That we can then measure the number of cells from this deterministic model.

In the case where they are uniform constants we can solve the equation. In the case where they are uniform constants across uniform spatial domains then we can also solve the equations evoking boundary conditions.

We now want to expand this model to include multiple malignant cell types. Also we want to include their stochastic dynamics as well.

3 MARKOV MODELS

Consider a cell with five possible mutations. We show the genes below. The call may begin with one mutation and then move to a second and so forth. Each path is assumed to be possible and the results of each path are different.



Now we can consider a model for the above simple example. We have 5 possible mutations and they may occur in any order. We assume they occur one at a time. We can identify any number of cells as:

$$n_{1,k}(x,t)$$

As the number of cells after one mutation at location x and at time t, of mutation k.

3.1 THE MARKOV ASSUMPTIONS

Now we have the following observations:

1. At mutation 1 we have 5 possible cell mutants. Furthermore each may be considered a cancer cell and the growth, diffusion and flow are as described above. Some of the mutations may be indolent and some aggressive.

2. At mutation 2 we have 5*4 possible cells. The question is that some are say PTEN then cMyc or cMyc then PTEN. Are they the same, and this means the difference between perturbation and

combination? Are they distinct by have been ordered differently or are they the same? If it is a combination we have 10 instead of 20 different mutations.

3. At mutation 3 we have 5*4*3 and at 4 we have 5*4*3*2 or 120 permutations.

4. At any location we may have any one or a combination of these mutation types. There are two factors driving their number:

a. A single type will have growth, dispersion and movement dynamics with the above mentioned model but each mutation will respond differently since their coefficients will be different. Some may grow faster and some may diffuse faster. There is no a priori ranking of the coefficients.

b. The surrounding mutant types will also tend to mitigate growth.

Now we can call the mutant cells as follows:

$$n_{1,k}(x,t)$$
$$n_{2,j,k}(x,t)$$
$$n_{3,i,j,k}(x,t)$$
$$n_{4,h,i,j,k}(x,t)$$
$$n_{5,g,h,i,j,k}(x,t)$$

Now we know how the subscripts can be ordered as per the above conversation. But we also assume that there exist some Markov mode for transitions from a 1 state to a 2 state, namely from one mutation to 2 mutations. That is we can assume a discrete time discrete state system and ascribe a Markov model with transition probabilities. Namely:

$$P\left[x(k+1)=x_0 \mid x(k)=x_0\right]=p_0$$
$$P\left[x(k+1)=x_{1,k} \mid x(k)=x_0\right]=p_{k,0}$$

Where we have the standard closure conditions on the transition probabilities. The process is Markov and it depends solely upon the prior state and no other.

In general we have:

$$P\left[x(k+1)=x_{n,k} \mid x(k)=x_{n,k}\right]=p_{n,k}$$
$$P\left[x(k+1)=x_{n+1,m} \mid x(k)=x_{n,k}\right]=p_{n:m,k}$$

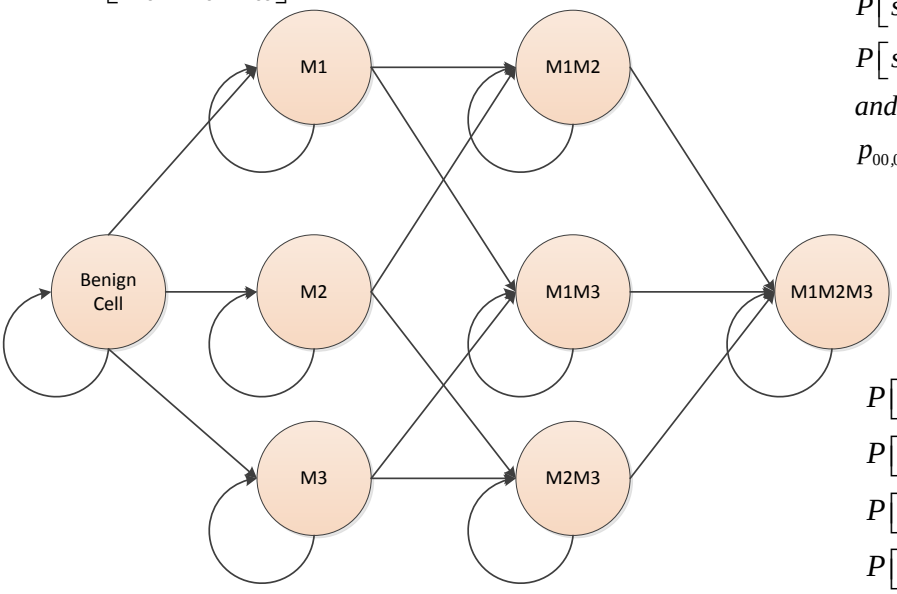
We can extend this to a continuous time system simply. We just need the mutation rates.

Now the initial equation describing single mutant growth was deterministic. However we now have demonstrated a random process. Thus we want to determine the average number at any time and at any location. The average will include the temporal Markovian dynamics of cell mutation rates which themselves may be spatially dependent.

3.2 THE MARKOV EXAMPLE

For example consider the following three gene mutation case:

$$P = \begin{bmatrix} p_{11} \cdots p_{21} \cdots p_{81} \\ \cdots \\ p_{18} \cdots p_{28} \cdots p_{88} \end{bmatrix}$$



$$P\left[s00 \middle| s00\right] = p_{00,00}$$
$$P\left[s11 \middle| s00\right] = p_{11,00}$$
$$P\left[s12 \middle| s00\right] = p_{12,00}$$
$$P\left[s13 \middle| s00\right] = p_{13,00}$$
and
$$p_{00,00} + p_{11,00} + p_{12,00} + p_{13,00} = 1$$

$$P\left[s11 \middle| s11\right] = p_{11,11}$$
$$P\left[s21 \middle| s11\right] = p_{21,11}$$
$$P\left[s22 \middle| s11\right] = p_{22,11}$$
$$P\left[s23 \middle| s11\right] = p_{23,11}$$
and
$$p_{11,11} + p_{21,11} + p_{22,11} + p_{23,11} = 1$$

Now for this simple example we can assume that 1 is the start and 8 is the end. We also assume that 2, 3, 4 are the first steps and 5, 6, 7 are the second. Then we have a transition probability matrix P as follows:

$$P = \begin{bmatrix} p_{1,1}..p_{2,1}..p_{2,2}..p_{2,3}..0.....0.....0.....0 \\ 0.....p_{2,2}..p_{2,5}..p_{2,6}..p_{2.7}..0.....0.....0 \\ 0.....0.....p_{3,3}..p_{3,5}..p_{3,6}..p_{3,7}..0.....0 \\ 0.....0....0.....p_{4,4}..p_{4,5}..p_{4,6}..p_{4,7}.....0 \\ 0.....0....0.....0.....p_{5,5}..0.....0.....p_{5,8} \\ 0.....0....0.....0.....0.....p_{6,8}.....0.....p_{6,8} \\ 0.....0....0.....0.....0.....0.....p_{7,8}....p_{7,8} \\ 0.....0....0.....0.....0.....0.....0.....p_{8,8} \end{bmatrix}$$

Where the sum across any row is unity. Thus we have:

$$p(k+1) = Pp(k)$$

where

$$p(k) = \begin{bmatrix} p_1(k) \\ ... \\ p_8(k) \end{bmatrix}$$

In this specific example.

Where p is the probability vector of all possible states, say 8 in our previous example, and P is the transition probability matrix. Note we have assigned P as dependent upon x and t. The same holds true for p.

Let us consider a simple example of N possible states and we examine the probability density of a specific state, Let:

$$p(n_i; x.t)$$

Where p is the probability density function of the random variable n on gene mix i. We assume that n is a continuous random variable so we can provide a simpler representation.

Now we can move to a continuous time simply by stating:

$$\frac{dp(x,t)}{dt} = P(x,t)p(x,t)$$

However we can simplify this as follows:

$$\frac{dp(n_i;x,t)}{dt} = -\lambda_{ii}\,p(n_i;x,t) + \sum_{j=1,j\neq i}^{N} \lambda_{i.j}\,p(n_j;x,t)$$

This is the continuous version of the above and is a simple Markov birth-death type model.

Now consider any point x, t in space-time. We can define:

$$E\left[n_i(x,t)\right] = \int n_i(x,t)\,p(n_i;x,t)\,dn_i$$
$$= \overline{n_i(x,t)}$$

We shall use this in the next section.

4 COMBINING

We now will combine the propagation equation model for a single malignant state with the overall evolution of malignant states via a Markov model.

Now consider a specific cell count at some specific x and t. We know we have:

$$\frac{\partial n_k(x,t)}{\partial t} = Ln_k(x,t)$$

Where L is the spatial operator.

We also have for the probability of each n being a specific value at a specific x and t as:

$$\frac{\partial p_k(x,t)}{\partial t} = \sum_{n=1}^{N} \lambda_n(x,t) p_n(x,t)$$

Now we can define the average of any n as follows:

$$\overline{n_k(x,t)} = \sum p_k(n_k(x,t) = n_m) n_m(x,t)$$

This is the average value of that specific n value for that specific gene mutation at that specific time and location. We have a means to calculate each of the probabilities and we then just calculate the average.

Now we can combine the equations as follows:

$$\sum p_k(x,t) \frac{\partial n_k(x,t)}{\partial t} = \sum \lambda_m(x,t) p_m(x,t) Ln_k(x,t)$$

or

$$\frac{\partial \sum p_k n_k}{\partial t} = L \sum \lambda_{m,k}(x,t) p_{m,k}(x,t) n_k(x,t)$$

or

$$\frac{\partial \overline{n_k(x,t)}}{\partial t} = L \lambda_k \overline{n_k(x,t)}$$

Let me redo the notation again. We will assume that n can be continuous for notation purposes. Thus we have:

$$\overline{n_k(x,t)} = \int p_k(n_k; x,t) n_k(x,t) dn_k$$

Now let us remember that for N possible n types we have:

$$\frac{dp_k(u_k;x,t)}{dt} = \sum_{j=1}^{N} \lambda_{k,j} p_j(u_j;x,t)$$

We have N equations like this.

If we multiply the propagation equation for any n as follows:

$$\frac{p_k(n_k;x,t)\partial n_k(x,t)}{\partial t} = p_k(n_k;x,t) L n_k(x,t)$$

Now introduce the integral:

$$\int p_k(n_k;x,t)\frac{\partial n_k(x,t)}{\partial t} dn_k = \int p_k(n_k;x,t) L n_k(x,t) dn_k$$

But recall that we can write:

$$\frac{\partial pn}{\partial t} = p\frac{\partial n}{\partial t} + n\frac{\partial p}{\partial t}$$

Then using what we know of p we have:

$$\frac{\partial p_k(n_k;x,t)}{\partial t} = -\lambda_{k,k} p_k(n_k;x,t) + \sum_{j=1;j\neq k}^{N} \lambda_{k,j} p_j(n_j;x,t)$$

Substituting and rearranging:

$$p_k(n_k;x,t)\frac{\partial n_k(x,t)}{\partial t} = \frac{\partial[p_k(n_k;x,t) n_k(x,t)]}{\partial t} - n_k(x,t)\frac{\partial p_k(n_k;x,t)}{\partial t}$$
$$= \frac{\partial[p_k(n_k;x,t) n_k(x,t)]}{\partial t} - n_k(x,t)\lambda_{k,k} p_k(n_k;x,t) + n_k(x,t)\sum_{j=1;j\neq k}^{N} \lambda_{k,j} p_j(n_j;x,t)$$

Now we can show that when we integrate to obtain averages we have:

$$\int p_k(n_k;x,t)\frac{\partial n_k(x,t)}{\partial t} dn_k = \frac{\partial \overline{n_k(x,t)}}{\partial t} - \lambda_{k,k}\overline{n_k(x,t)} - \sum_{j=1;j\neq k}^{N} \lambda_{k.j} n_j(x,t)$$

Thus we can replace this in the propagation equation to read:

$$\frac{\partial \overline{n_k(x,t)}}{\partial t} - \lambda_{k,k}\overline{n_k(x,t)} - \sum_{j=1;j\neq k}^{N} \lambda_{k.j} n_j(x,t) = L\overline{n_k(x,t)}$$

Now if we had multiplied by the joint pdf we would have obtained:

$$\frac{\partial \overline{n_k(x,t)}}{\partial t} - \lambda_{k,k}\overline{n_k(x,t)} - \sum_{j=1;j\neq k}^{N} \lambda_{k.j} \overline{n_j(x,t)} = L\overline{n_k(x,t)}$$

Or; upon rearranging we obtain:

$$\frac{\partial \overline{n_k(x,t)}}{\partial t} = L_k\overline{n_k(x,t)} - \lambda_{k,k}\overline{n_k(x,t)} + \sum_{j=1;j\neq k}^{N} \lambda_{k.j} \overline{n_j(x,t)}$$

Thus the result for the average is a set of linked partial differential equations. Note we have modified the L operator to reflect specificity for k. The added terms reflect the movement of cell types from one class to another.

This is a powerful equation. It tells us how specific cells diffuse, flow and reproduce, and then how they migrate to new types of cells.

Let us take it one step further. Recall:

$$L_k = a_k \frac{\partial^2}{\partial x^2} + b_k \frac{\partial}{\partial x} + c_k$$

define

$$\widetilde{L_k} = L_k - \lambda_{k,k}$$

Now consider a vector of all n possibilities and we can determine the average vector of these as follows:

$$n(x,t) = \begin{bmatrix} n_1(x,t) \\ \dots \\ n_N(x,t) \end{bmatrix}$$

And where the average of the vector is the average of the above. Then we readily have the equation for all n as follows:

$$\boxed{\frac{\partial \overline{n(x,t)}}{\partial t} = \widetilde{L}\overline{n(x,t)} + \Lambda\overline{n(x,t)}}$$

Where:

$$\tilde{L} = \left[\tilde{L}_1 ..... \widetilde{L_N} \right]$$

and

$$\Lambda = \begin{pmatrix} -\lambda_{11} & \lambda_{12} & \lambda_{13} \\ \lambda_{21} & -\lambda_{22} & \lambda_{23} \\ \lambda_{31} & \lambda_{32} & -\lambda_{33} \end{pmatrix}$$

The above is suggestive and it depends on the specific model.

5 OBSERVATIONS

To summarize the following depicts the major analytical results:

Propagation Model: This equation provides a spatio-temporal model for the calculation of the number of specific cancer cells which are propagated by means of: (i) diffusion, (ii) flow, and (iii) proliferation.

$$\frac{\partial n(x,t)}{\partial t} = a\frac{\partial^2 n(x,t)}{\partial x^2} + b\frac{\partial n(x,t)}{\partial x} + cn(x,t)$$

Average Model: This model considers the calculation of the average number of malignant cells in a spatio-temporal manner when the cells mutate into N possible genetic variants. It calculates the average number by variant and thus is a vector equation containing the N variants.

$$\frac{\partial \overline{n(x,t)}}{\partial t} = \tilde{L}\overline{n(x,t)} + \Lambda\overline{n(x,t)}$$

We can thus make several important observations regarding this model.

1. Prognostic and Therapeutic: We can determine the transitions and the factors related to diffusion, flow and growth. Thus we can use the result as a powerful one for prognostic and therapeutic results. As we had indicated earlier, the Observability and Controllability issues are essentially Prognostic and Therapeutic respectively.

2. Variances: The results are for the average. We can determine the results for the variances as well. We have examined the variances on the averages and they are somewhat complex and we do not believe that they lend significant additional information at this time.

3. Solutions: The solutions to these equations are readily obtained using standard techniques. They can, in addition, be determined in closed form results.

5.1 MEASURING THE PARAMETERS

Measuring the parameters in these models has been discussed before for a single mutation. However, we have now introduced a set of multiple and progressive mutations. How do we know how these mutations progress? Can the mutation progressions be determined a priori or do

they occur in some random fashion? How do we deal with the epigenetic elements such as hypermethylation and miRNAs when we consider changes in expression without mutations?

There are a significant number of questions that we must consider when examining the change in gene expression. In addition the environment, extracellular matrix as well as surrounding cells may also effect changes in gene expression.

Thus ascertaining gene mutation or expression would most likely be determined by examining the cells expression themselves. At the current time there does not appear to be a robust theory which can be used as a basis for such projections. We are left measuring what has actually happened rather than projecting what will occur.

Perhaps subsequent Bayesian analysis will allow for such determination.

5.2 IN SITU HEMATOLOGICAL MEASUREMENTS

The challenge is determining of a cancer has metastasized is to find out where and how much. The classic approach is to look at the local draining lymph nodes and see if has gone there. However the cancer cells may often escape through the blood system and not the lymph system. Consider ocular melanoma, there is no lymph system connection and it spreads by hematological means only.

That means that by examining the blood we should be able to find the wandering malignant cells, at least in theory. In a recent release by *MedGadget* the article relates developments at MGH in Boston as follows[2]:

Circulating tumor cells (CTCs) are shed by primary tumors and allow the cancer to metastasize to the distant sites. While this is a devastating tool in cancer's war chest, it offers clinicians a marker through which to diagnose and monitor progress of the disease. Since the discovery of CTCs over a hundred years ago, researchers have been developing ever more sensitive methods of capturing them since they're extremely rare in whole blood.

In a recent development by Ozkumur et al at MGH[3] the authors' state:

Circulating tumor cells (CTCs) are shed into the bloodstream from primary and metastatic tumor deposits. Their isolation and analysis hold great promise for the early detection of invasive cancer and the management of advanced disease, but technological hurdles have limited their broad clinical utility. We describe an inertial focusing–enhanced microfluidic CTC capture platform, termed "CTC-iChip," that is capable of sorting rare CTCs from whole blood at 107 cells/s.

[2] http://www.medgadget.com/2013/04/mgh-ctc-ichip-sets-new-bar-for-circulating-tumor-cell-detection.html

[3] http://stm.sciencemag.org/content/5/179/179ra47

Most importantly, the iChip is capable of isolating CTCs using strategies that are either dependent or independent of tumor membrane epitopes, and thus applicable to virtually all cancers. We specifically demonstrate the use of the iChip in an expanded set of both epithelial and nonepithelial cancers including lung, prostate, pancreas, breast, and melanoma.

The sorting of CTCs as unfixed cells in solution allows for the application of high-quality clinically standardized morphological and immunohistochemical analyses, as well as RNA-based single-cell molecular characterization. The combination of an unbiased, broadly applicable, high-throughput, and automatable rare cell sorting technology with generally accepted molecular assays and cytology standards will enable the integration of CTC-based diagnostics into the clinical management of cancer.

There are several problems here however:

1. As we had demonstrated in some of our prior analysis, blood borne cancer cells are rare, but more importantly they are cells which are coming from and going to organs. Namely they are in transit, from whence and to where we do not know.

2. The genetic states of each of these wandering cells may be a marker of from whence it came. The problem is that we do not fully understand this genetic mutation process, and in fact as we have shown before it may actually be a Markov like chain process.

3. Understanding this change in cells may be of significant therapeutic value. However this again is uncertain given our current state of knowledge.

4. Again we come back to the cancer stem cell and ask if the few cells we find in the blood stream are the right cells to examine.

However this advance could provide significant data to allow us to expand the understanding of mutating cancer cells.

It seems that there is a significant amount of new work being done on evaluating cancers via circulating tumor cells and their DNA. Another paper in Nature states:

Cancers acquire resistance to systemic treatment as a result of clonal evolution and selection. Repeat biopsies to study genomic evolution as a result of therapy are difficult, invasive and may be confounded by intra-tumour heterogeneity Recent studies have shown that genomic alterations in solid cancers can be characterized by massively parallel sequencing of circulating cell-free tumour DNA released from cancer cells into plasma, representing a non-invasive liquid biopsy.

Here we report sequencing of cancer exomes in serial plasma samples to track genomic evolution of metastatic cancers in response to therapy. Six patients with advanced breast, ovarian and lung cancers were followed over 1–2 years. For each case, exome sequencing was performed on 2–5 plasma samples (19 in total) spanning multiple courses of treatment, at

selected time points when the allele fraction of tumour mutations in plasma was high, allowing improved sensitivity.

For two cases, synchronous biopsies were also analysed, confirming genome-wide representation of the tumour genome in plasma. Quantification of allele fractions in plasma identified increased representation of mutant alleles in association with emergence of therapy resistance. ...treatment with gefitinib.

These results establish proof of principle that exome-wide analysis of circulating tumour DNA could complement current invasive biopsy approaches to identify mutations associated with acquired drug resistance in advanced cancers. Serial analysis of cancer genomes in plasma constitutes a new paradigm for the study of clonal evolution in human cancers.

Cancer Research UK commented on the works as follows[4]:

Scientists ... used traces of tumour DNA, known as circulating tumour DNA (ctDNA) found in cancer patients' blood to follow the progress of the disease as it changed over time and developed resistance to chemotherapy treatments.

They followed six patients with advanced breast, ovarian and lung cancers and took blood samples, which contained small amounts of tumour ctDNA, over one to two years.

By looking for changes in the tumour ctDNA before and after each course of treatment, they were able to identify which changes in the tumour's DNA were linked to drug resistance following each treatment session.

Using this new method they were able to identify several changes linked to drug-resistance in response to chemotherapy drugs such as paclitaxel (taxol) which is used to treat ovarian, breast and lung cancers, tamoxifen which is used to treat oestrogen-positive breast cancers and transtuzumab (Herceptin) which is used to treat HER2 positive breast cancers.

And they hope this will help shed new light on how cancer tumours develop resistance to some of our most effective chemotherapy drugs as well as providing an alternative to current methods of collecting tumour DNA – by taking a sample direct from the tumour – a much more difficult and invasive procedure.

As we noted in a previous note regarding the same set of procedures by others researchers this is a useful method to detect the progression of cancer.

However the following observations are of note:

1. Are these coming or going cells, namely are the cells on their way to a metastasis or the result of one.

[4] http://www.cancerresearchuk.org/cancer-info/news/archive/pressrelease/2013-04-07-simple-blood-test-to-track-tumour-evolution?rss=true

2. Can we use these cells to determine the changes in DNA expression as the cells progress.

3. How effective a prognostic tool are these measurements.

4. What therapeutic methods can be applied now knowing this information.

Thus is this data of primary use or secondary. Notwithstanding its clinical use it does represent an excellent tool for genomic progression.

5.3 NON INVASIVE METHODOLOGIES

Molecular Functional Imaging, MFI, provides a set of non-invasive methodologies to ascertain pathway dynamics as well as changes in genetic expression[5]. As Glunde et al state:

Molecular–functional imaging (MFI) can be defined as the noninvasive visualization of molecular and functional pathways in the tissue of interest.

As Glunde et al state:

Molecular imaging of cancer detects single molecules or their activity within cancer cells in culture or within a solid tumor. These molecules can be overexpressed receptors, activated enzymes or relocated molecules, each of which plays important roles in signaling cascades or regulatory programs that are deregulated in cancer. These in turn give cancer its phenotypic characteristics, such as evasion of apoptosis, self-sufficiency in growth signals, insensitivity to anti-growth signals, sustained angiogenesis, unlimited replicative potential, invasion of tissue and metastasis.

They continue focusing on the ability with MFI to monitor pathway dynamics:

The ability to image gene expression, promoter activity, and transcriptional activity in vivo is important because these are the starting points for many deregulated pathways in cancer. Reporter genes that are typically used include luciferase genes for bioluminescence imaging, fluorescent-protein genes for fluorescence imaging, herpes simplex virus thymidine kinase (HSVtk) genes for PET and SPECT imaging and ferritin genes or chemical exchange saturation transfer (CEST) reporters for MRI. These reporter genes are placed under the control of a promoter of interest so that promoter activity in vivo can be evaluated.

Triple-fusion-reporter genes that allow for in vivo multi-modality imaging with bioluminescence, fluorescence and PET have recently been developed. Imaging gene expression has helped to delineate mechanistic and functional aspects of oncogenes, such as myc, and tumor suppressor genes, such as p53. Another important application of imaging gene expression is monitoring viral vector delivery in vivo for future gene therapies.

[5] Further consideration of the material in this section was provided by discussions with Dr. Annick Van den Abbeele at Dana Farber Cancer Institute, Boston, MA.

5.3.1 Pathway Expression Estimation

Glunde et al discuss two specific genes; myc and p53. Let us first consider what they say about myc:

The myc oncogene is one of the most commonly activated oncogenes associated with the pathogenesis of liver cancer. In vivo bioluminescence imaging of transgenic mice conditionally expressing tetracycline-inducible myc proto-oncogene in liver cells proved that myc oncogene inactivation resulted in dormancy as long as myc remained inactive but that myc reactivation immediately restored the neoplastic features of previously differentiated hepatocytes and biliary cells. In this study, myc activation probably caused malignant expansion of immature liver cells with stem-cell like properties, supporting the hypothesis that liver tumors can arise from cancer stem cells.

The cancer stem cell issue is also a significant one in this analysis. We have discussed cancer stem cells previously and they are key factors in assessing metastatic behavior. However they are also of low density and generally difficult to describe genetically.

Glunde et al then progress to a discussion of p53:

The p53 tumor-suppressor gene, which is mutated in rv50% of all human cancers, plays a key role in cell cycle regulation and apoptosis following DNA damage by functioning as a sequence-specific transcription factor. Bioluminescence imaging was employed for the noninvasive evaluation of the transcriptional activity of p53 in vivo in a transgenic mouse model that conditionally expressed the firefly luciferase gene upon activation by a p53-responsive promoter. After exposure to ionizing radiation, the in vivo p53 transcriptional activity displayed a distinct oscillatory pattern, confirming p53 transcriptional oscillations previously observed in cultured cells. In vivo bioluminescence imaging of transgenic mice will prove useful in future studies assessing p53 response in vivo after systemic administration of novel therapeutic p53 or proteasome inhibitors or of agents modulating the response to ionizing radiation.

The following Table is an adaptation from the paper summarizing some targets for MFI:

Cancer characteristics/pathways	Molecular/cellular targets in cancer	Molecular MFI applications
Oncogenesis pathways	p53	Bioluminescence, PET
	myc	Bioluminescence
Multidrug resistance	P-glycoprotein	PET, SPECT
Apoptosis	Phosphatidyl serine	PET, SPECT, MRI, Optical
	externalization	(Annexin V)
Cell surface receptors	EGFR	PET, SPECT, NIR fluorescence
	HER-2/neu	T1-MRI, T2-MRI, PET, SPECT,
		fluorescence
	PSMA	PET, SPECT, fluorescence
Proliferation/differentiation	Thymidine kinase	PET (^{18}F-FLT)

	Telomerase	PET
Angiogenesis/lymph-	VEGF	PET, SPECT, MRI, fluorescence
angiogenesis		
Hypoxia	avb3 HIF-1	Fluorescence, PET, SPECT, MRI PET, fluorescence
Metabolism	NA	NA
ECM degradation	Cathepsin D	NIR fluorescence
	Cathepsin B	NIR fluorescence
	Matrix	NIR fluorescence
	metalloprotease 2	
	Lysosomes	Fluorescence
Invasion and metastasis	Cell labeling with	Fluorescence
	fluorescent proteins	

5.3.2 ECM Imaging

We have discussed the impact of the extracellular environment for melanoma in previous sections and thus being able to deal with that is critical as well. Thus as Glunde et al sate:

A growing list of imaging techniques such as differential interference contrast (DIC) microscopy, confocal reflection microscopy, second harmonic generation (SHG) microscopy, Fourier transform infrared (FTIR) micro-spectroscopy and atomic-force microscopy (AFM) (reviewed in) are helping us to understand the interaction be- tween tumor cells and the ECM. For example, Nomarski DIC optics has been used for dynamically tracking cell- induced matrix remodeling. Assembly properties of type I collagen and interstitial ECM have been studied with confocal reflection microscopy, without the need for staining the specimen. The nonlinear optical process of SHG requires an environment without a center of symmetry, such as an interfacial region, to produce a signal. SHG was proposed as a new contrast mechanism for live- cell imaging and was extended to image endogenous structural proteins such as those within collagen-rich layers. FTIR micro-spectroscopy, which probes the vibration energy of chemical bonds, has been used for imaging the proteolytic activity of matrix metalloproteinases (MMPs) produced by invasive cancer cells on collagen-based matrices

5.3.3 Next Steps

Glunde et al list the following next steps for MFI:

- *Further development of novel strategies to detect and image specific pathways and targets is required.*
- *The low concentration of receptors and molecular targets and the inherent insensitivity of imaging techniques, such as MRI, impose limitations, which require amplification strategies for increasing the sensitivity of detection.*

- *Intracellular access of reporter molecules or the internalization of reporters is necessary for imaging several critical pathways and molecules and presents another challenge.*
- *As the acquisition of multi-parametric and multi-modality images becomes increasingly routine, integrated multi-modal approaches will present unique technical and computational challenges as well as exciting opportunities.*
- *The limited translation of these approaches to clinical use presents the major challenge to date.*

The summary above clearly indicates a significant potential if the challenges are met but they also present a significant set of current limitations.

With molecular functional imaging it is possible to do the following:

1. Target specific pathway modalities so as to ascertain the state of the cells in any part of the body and to determine the mutation profile of the malignancy. Specifically we can determine the progression from the initial mutation to subsequent ones. It is suspected that mutation profiles will be of great assistance in both prognostic as well as therapeutic approaches.

2. Spatial progression will be able to be identified, quantified and projected. True assessment of metastatic movement can be made at the molecular cell level.

3. Temporal changes will be the most critical of measurements. We will be able to examine rates of change both in metastasis as well as in response to therapeutic advances.

MFI can provide a critically impressive capability which when combined with the modeling described herein will allow for improved prognostic capabilities as well as improved therapeutics.

6 REFERENCES:

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