The Tumor Microenvironment and 3-D Tumor Models

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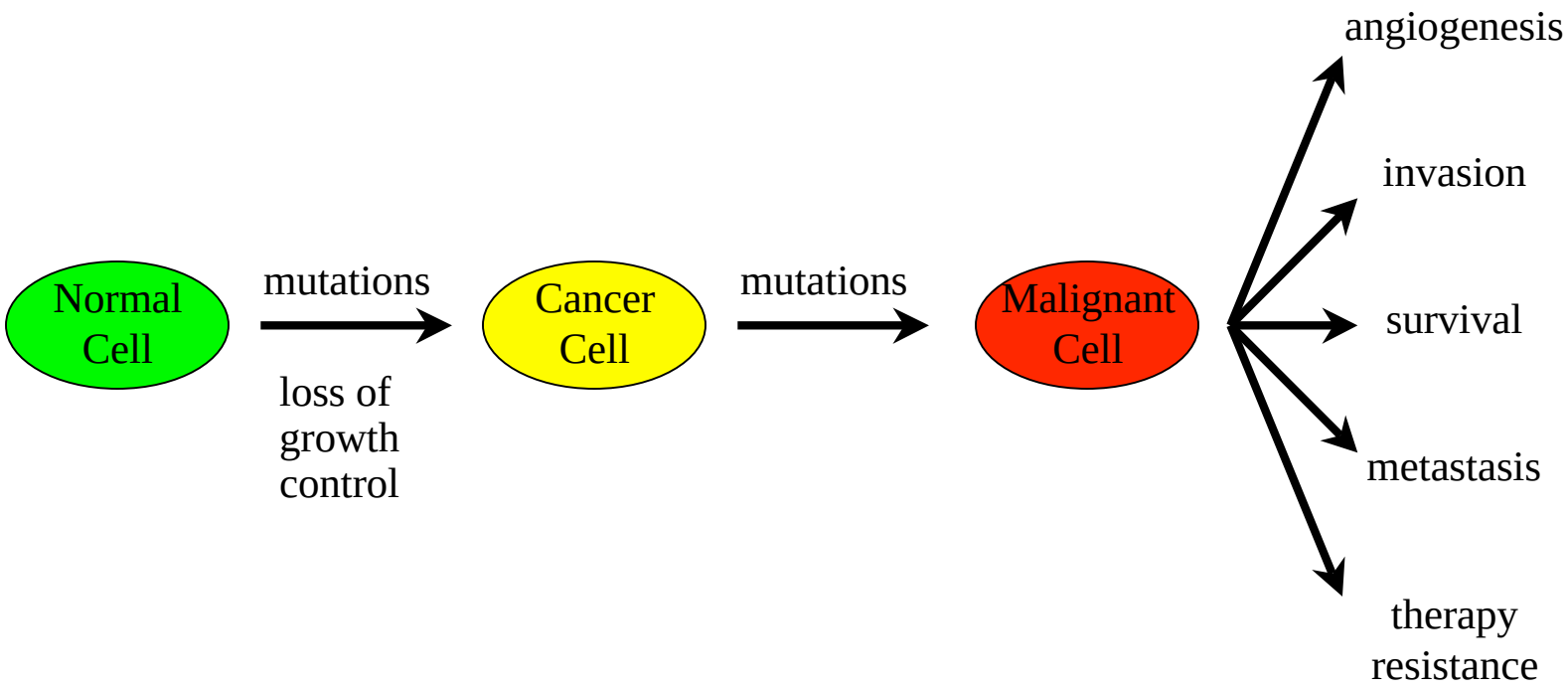
Outline

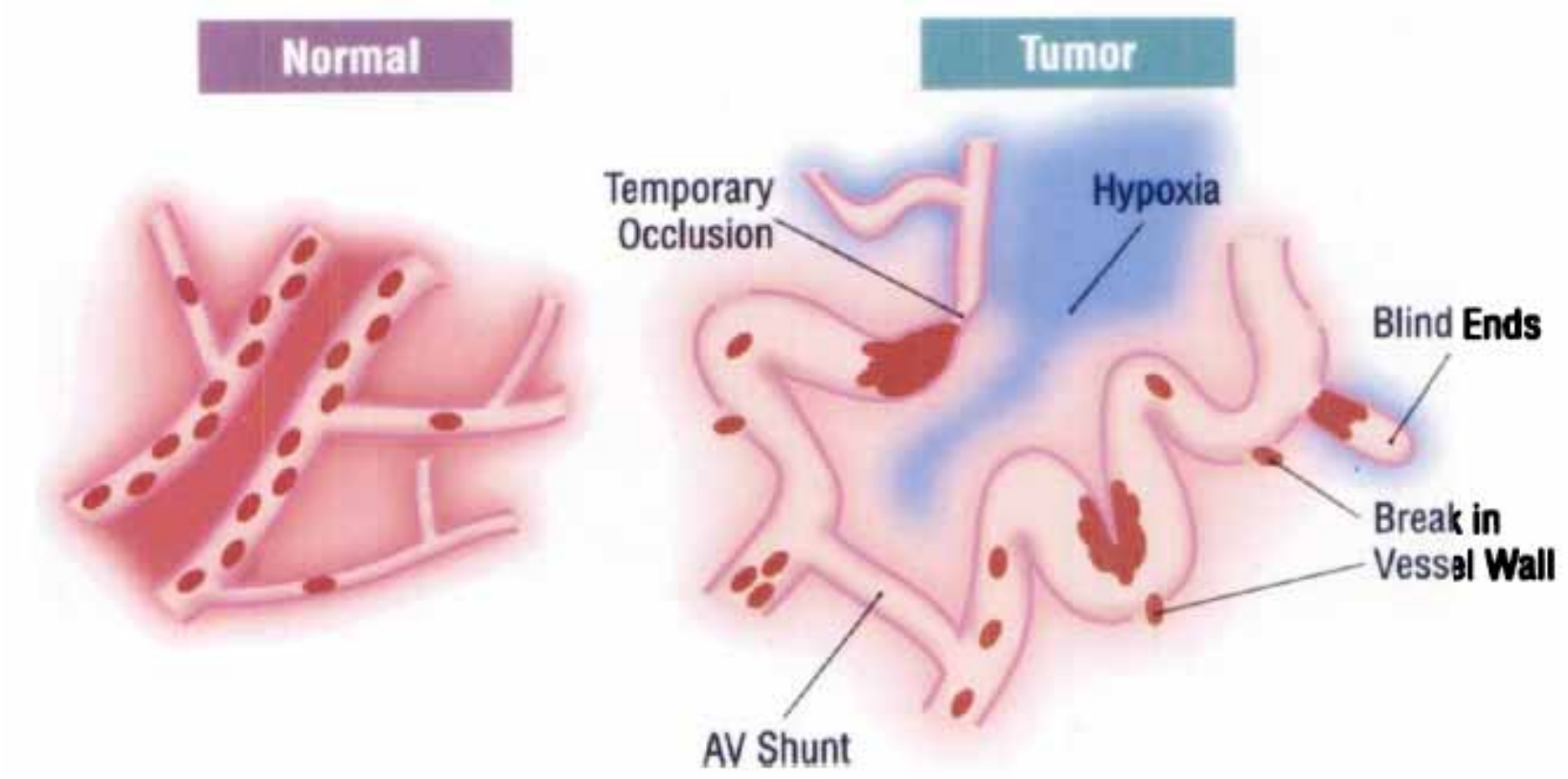
- The Tumor Microenvironment
  - Chronic versus acute changes
  - Consequences of tumor microenvironment
  - Advances in measuring the tumor microenvironment
  - Difficulties with *in vivo* models and clinical tumors
- 3-D Experimental Tumor Model Systems
  - Types of model systems
  - The multicellular spheroid tumor model
  - Example of application of spheroids
  - Recent developments and future work
- Mathematical Modeling in Tumor Biology
  - Tumor microenvironment
  - Genetic/proteomic/metabolic networks
  - Tumor growth and development
- Questions?

Malignant Progression of Cancer



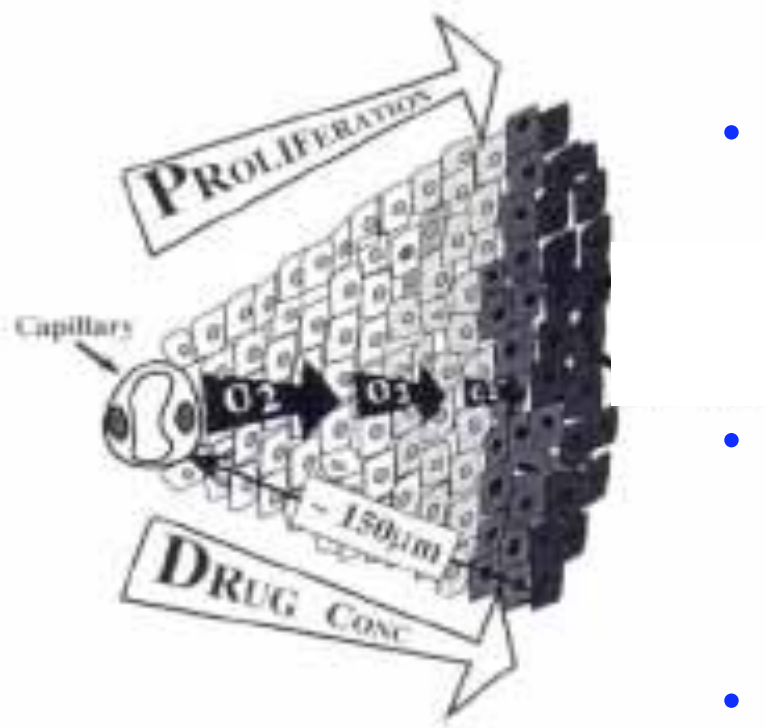
Important to realize: all of this happens in a 3-D context within a tissue!

Differences: Tumor and Normal Tissue Vasculature



Brown & Giaccia, *Cancer Res.* 58: 1408, 1998

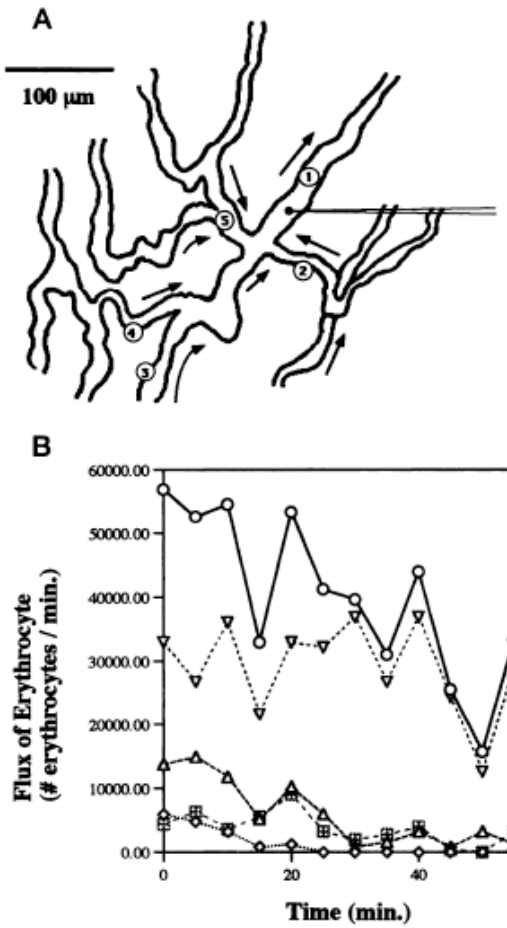
Chronic Changes in Tumor Microenvironment



- Tumor cells grow faster than vasculature: cells located far from vessels
- Gradients in biochemistry of extracellular space
  - Nutrients (oxygen, glucose)
  - Metabolic wastes (pH, lactate)
  - Signaling molecules (promotors, inhibitors)
- Gradients in cell physiology
  - Proliferation
  - Metabolism
  - Viability
  - Motility, invasiveness
- Gradients in gene/protein expression
- Gradients in therapy response
- Generally occur over ~200 μm

Brown & Giaccia., *Cancer Res.* 58: 1408, 1998

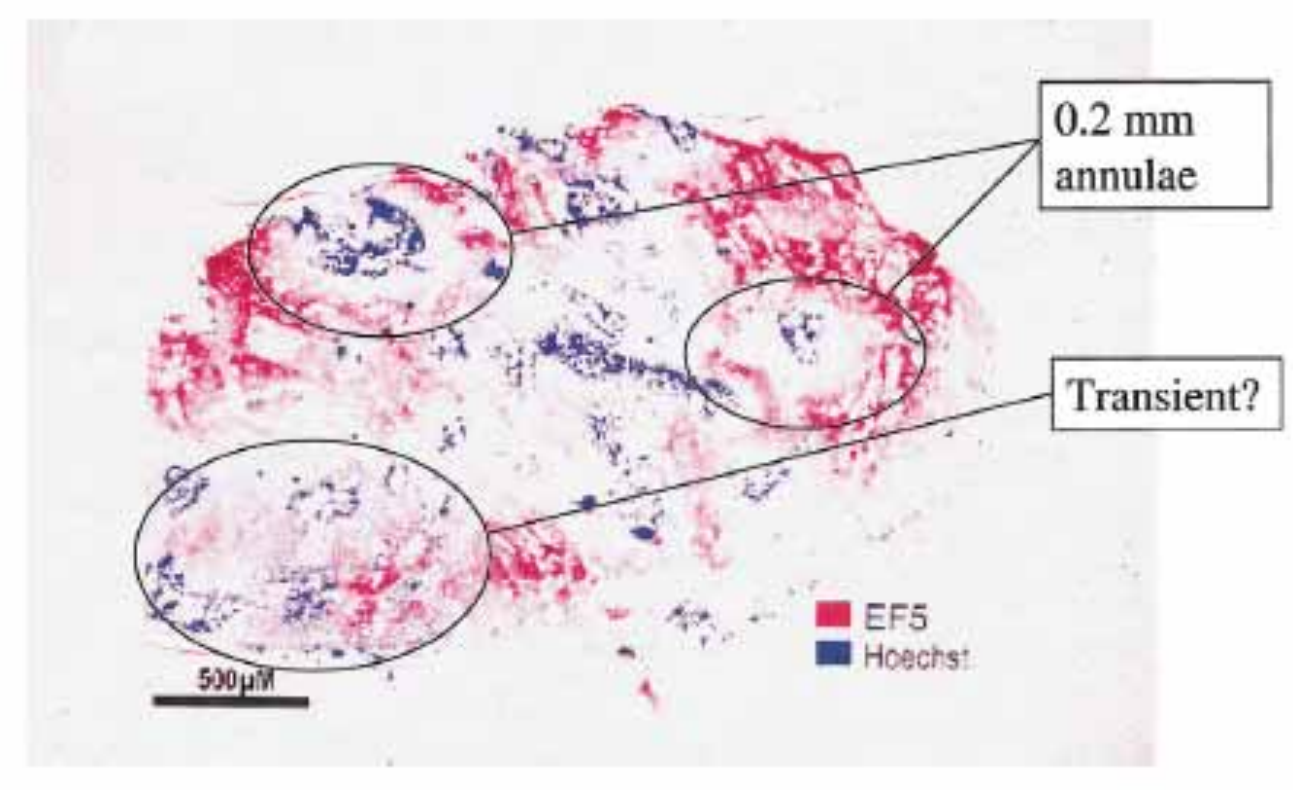
Transient Changes in Tumor Microenvironment



Kimura et al., *Cancer Res.* 56: 5522, 1996

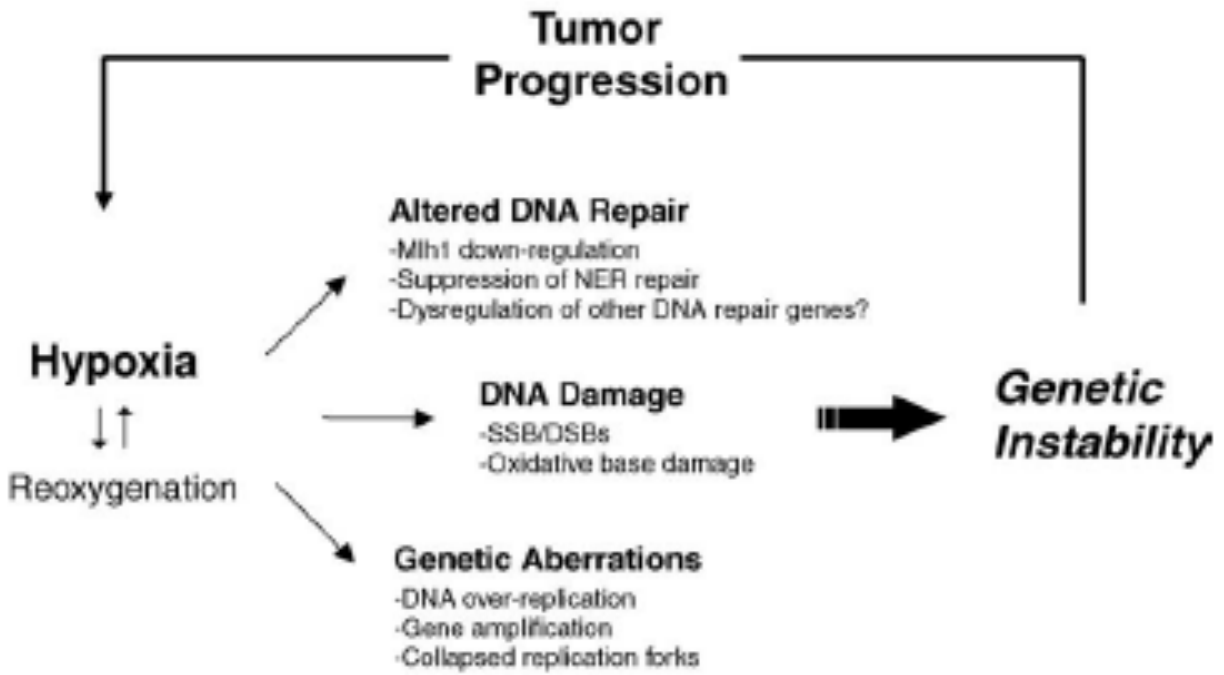
- No organization to architecture of vasculature: driven by semi-random processes
  - Long, tortuous vessels
  - A-V shunts
  - Blockages
- Disorganized function
  - No smooth muscle or nerve cells
  - Varying pressure gradients
  - Trapping of white/red cells
- Transient microregional variations in flow
  - Slowed, stopped, reversed flow
  - ~10-20 minute period most frequent
- Time-varying nutrient supply and waste removal
- Superimposed on chronic gradients
- Altered by therapy

Both Chronic and Transient Hypoxia



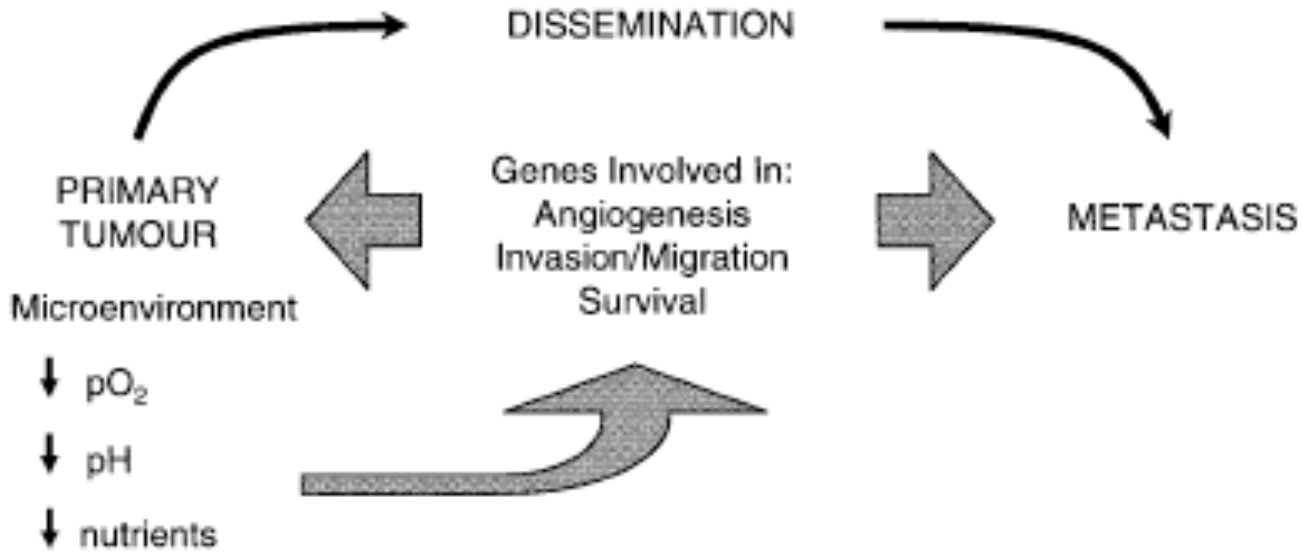
Gilles et al., *J. Magnet. Reson. Imag.* 16: 430, 2002

Microenvironment Involved in Tumor Progression



Bindra & Glazer., *Mutat. Res*. 569: 75, 2005

Microenvironment Involved in Metastasis

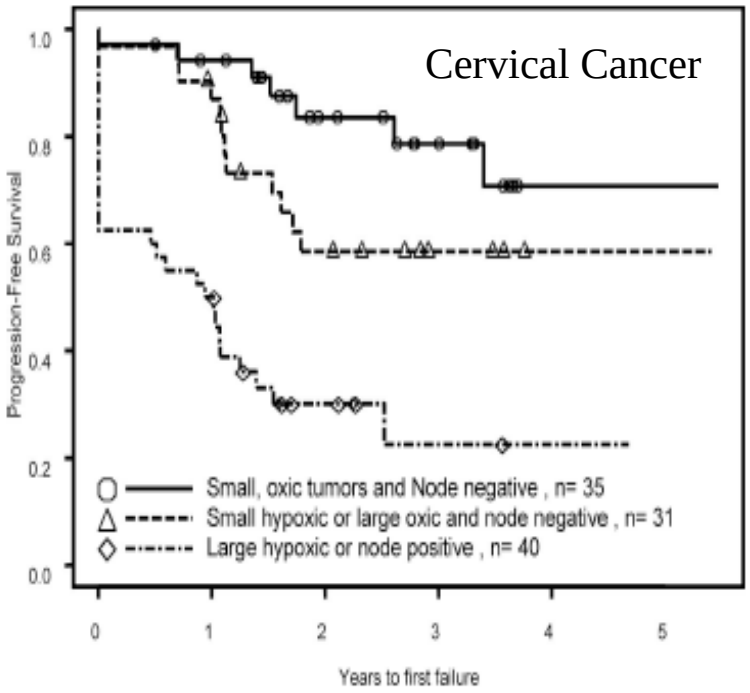


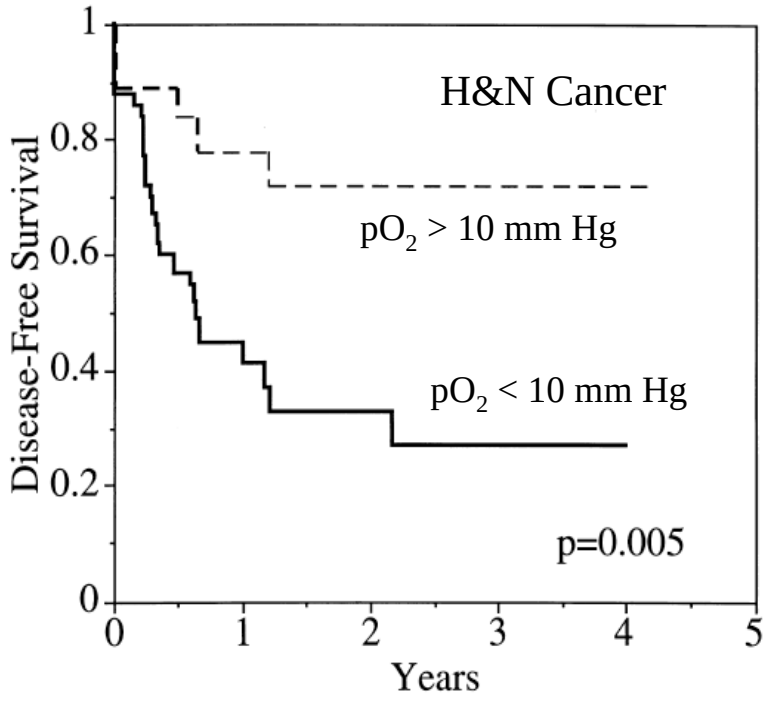
Sabarsky & Hill., *Clin. Exper. Metast.* 20: 237, 2003

Therapeutic Impact of Tumor Microenvironment

- Hypoxia causes radiation resistance
  - Major explanation for radiotherapy failure
  - Major focus of drug development and imaging
- Cell cycle arrested cells more resistant
  - Resistant to most common chemotherapies, radiation
  - Able to repopulate tumor after treatment
- Limited drug delivery
  - Poor penetration (chronic) & limited delivery (transient)
  - Problem for new therapies (antibodies, nonparticles)
- Induction of drug resistance and genetic instability
  - Gene expression and protein modifications
  - Mutations: drug resistance, survival phenotypes
- Stimulation of angiogenesis and metastatic spread
  - Induction of pro-angiogenic factors
  - Increased local invasion and distant metastases

Effect of Hypoxia on Therapy





Fyles et al., *J. Clin. Oncol.* 20: 680, 2000

Brizel et al., *Radiother. Oncol.* 53:113, 1999

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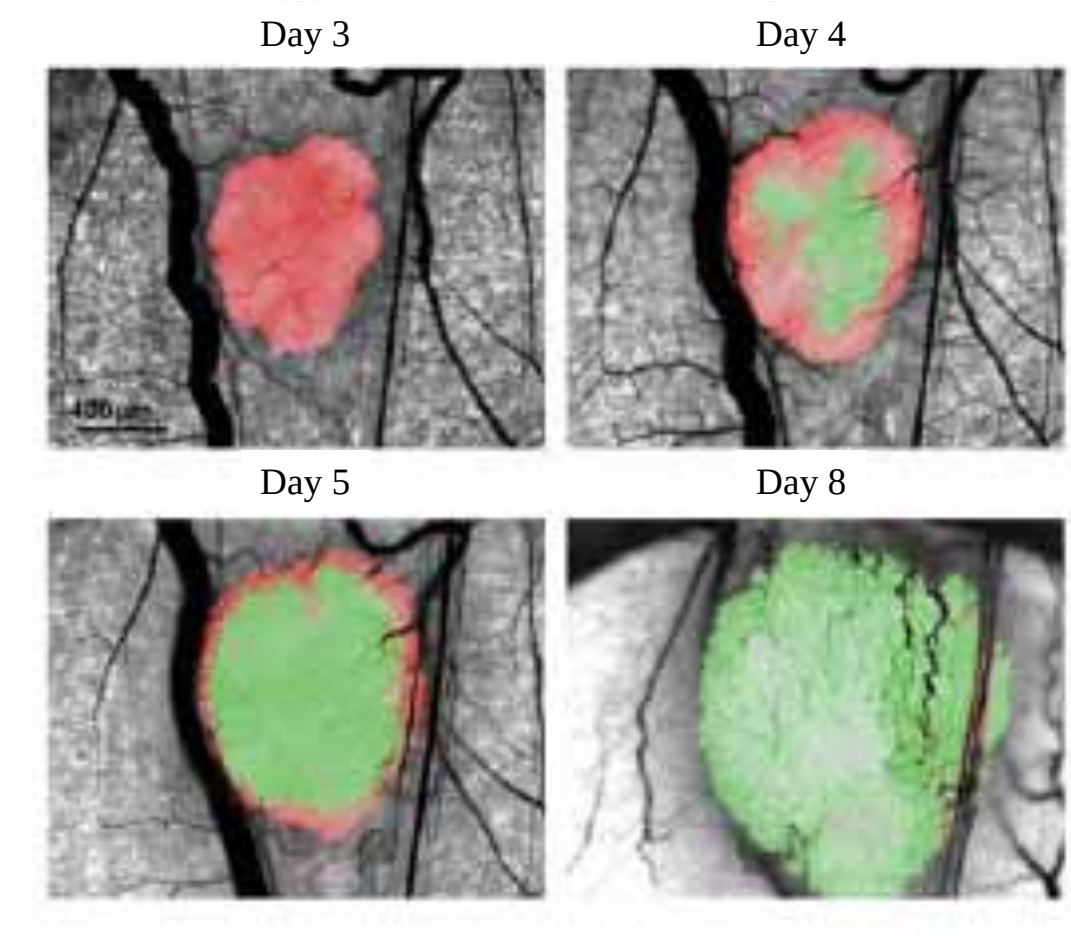
Imaging in Window Chamber Tumors

Oxygenated



Hypoxic

Sorg et al., *J. Biomed. Optics* 10: 044004, 2005

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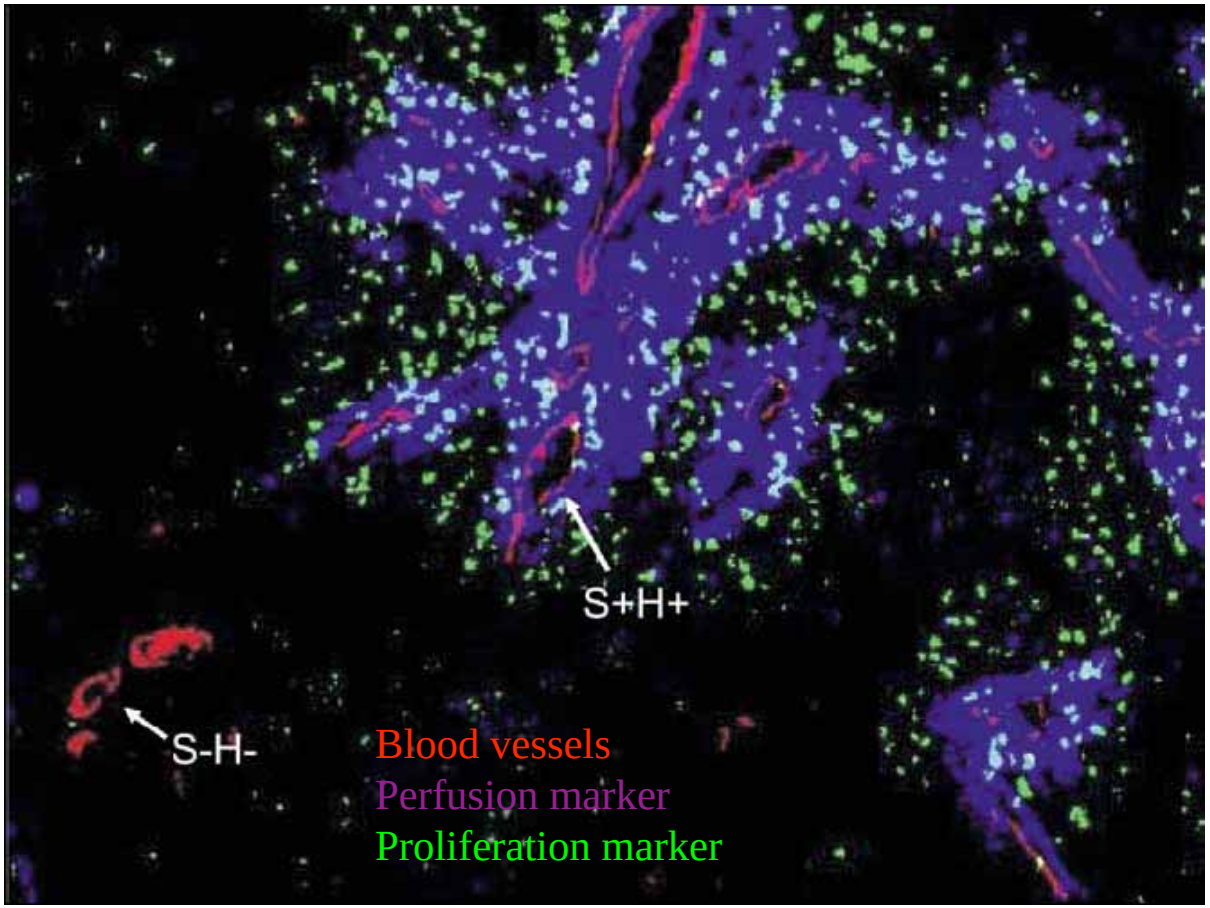
Imaging in Human Tumor Sections



Janssen et al., *Int. J. Radiat. Biol. Phys.* 62: 1169, 2005

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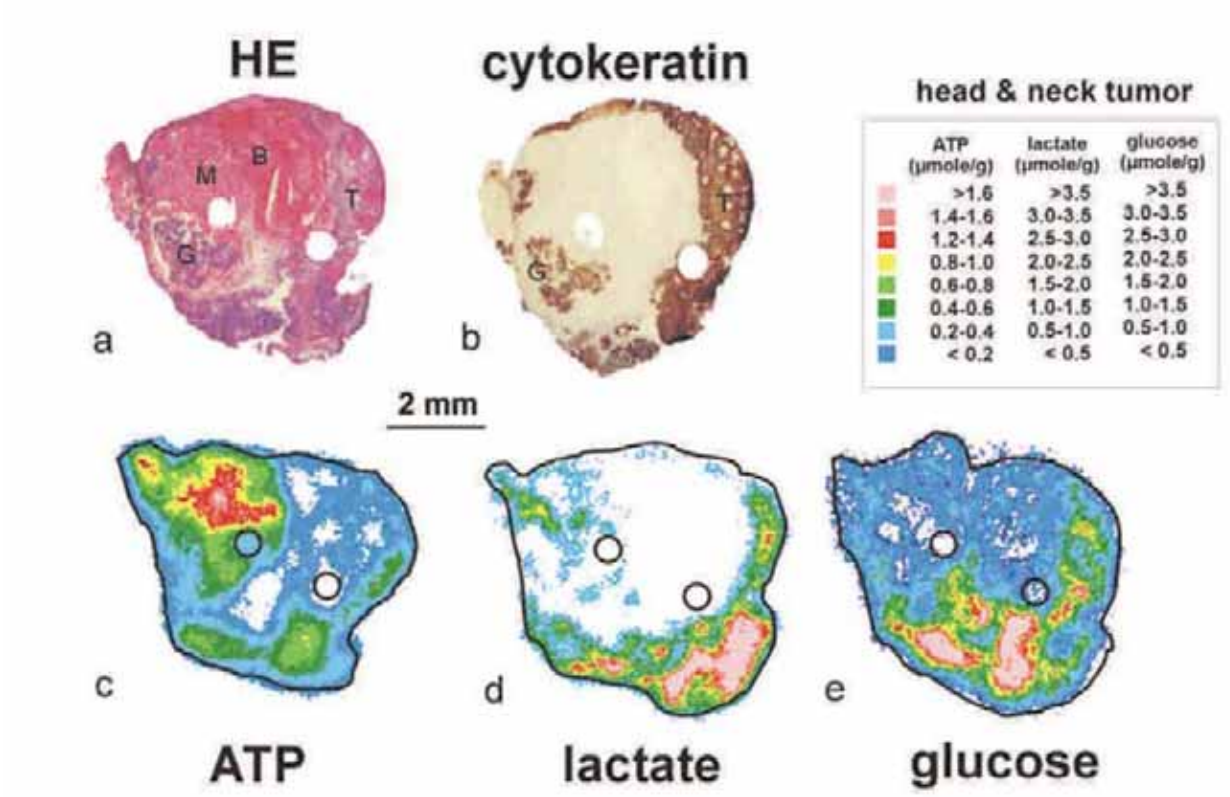
Metabolic Analysis of Tumor Microenvironment



Wallenta et al., *Biomol. Engineer*. 18: 249, 2002

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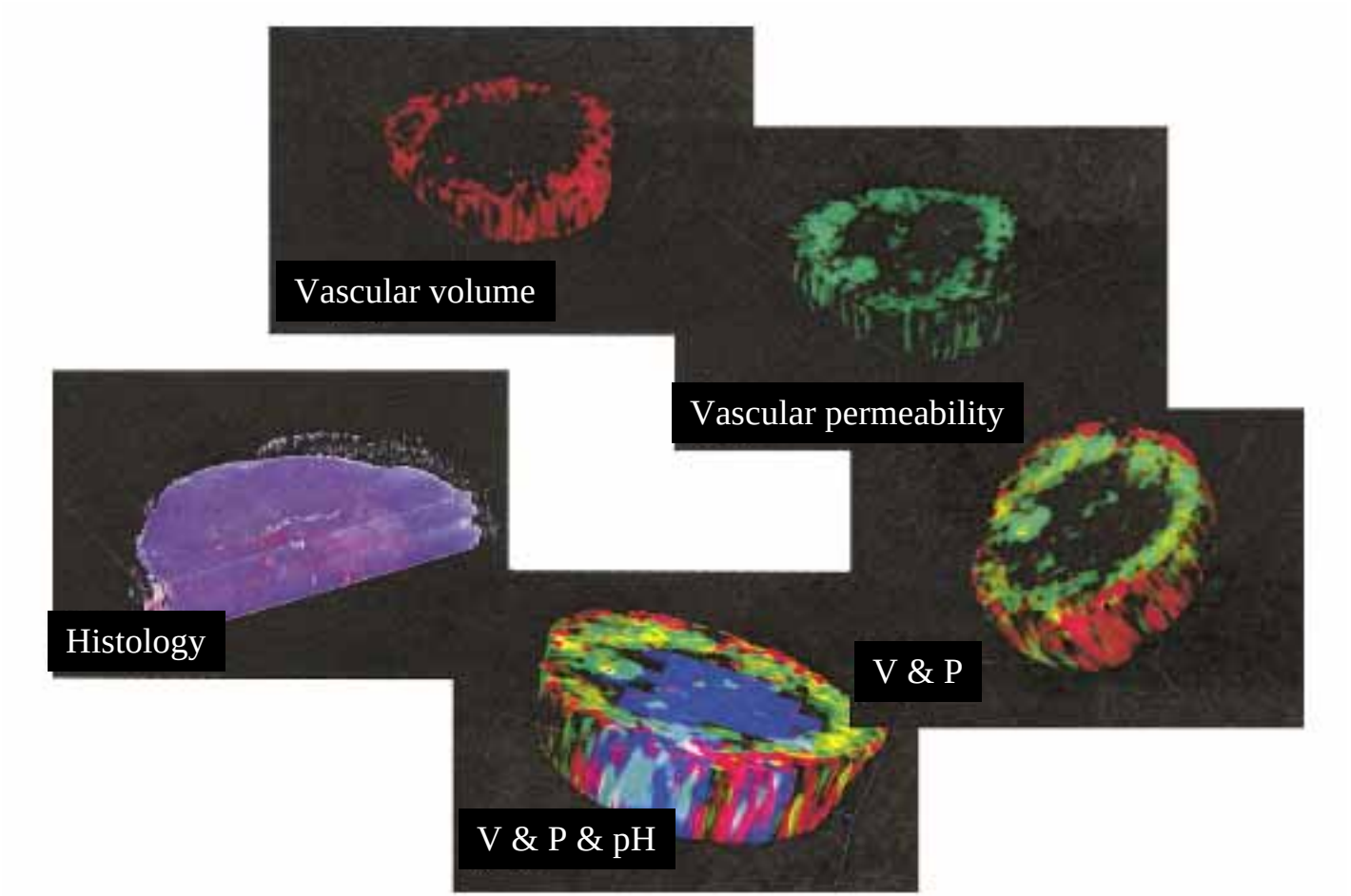
Advanced MRI of Tumor Microenvironment



Gilles et al., *J. Magnet. Reson. Imag.* 16: 430, 2002

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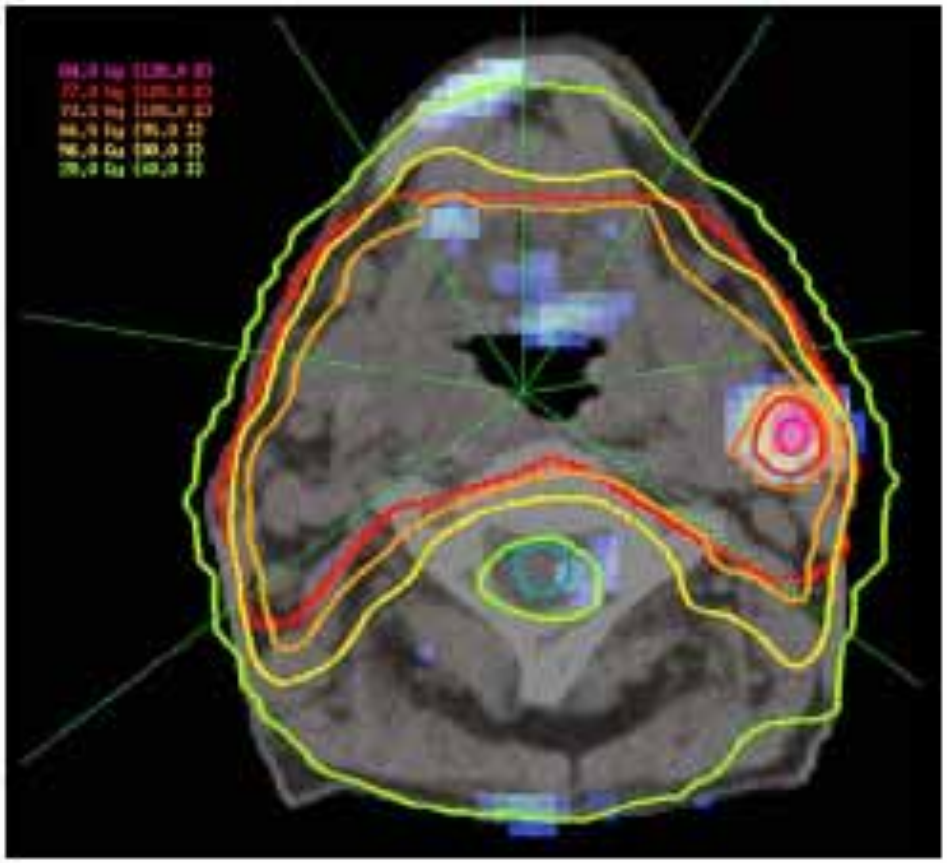
Advanced MRI of Human H&N Tumor



Padhani et al., *Eur. Radiol.* 17: 861, 2007

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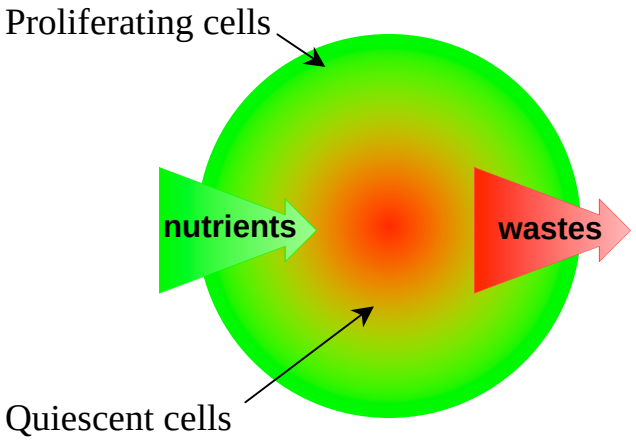
Limitations to *in Vivo* Tumor Biology

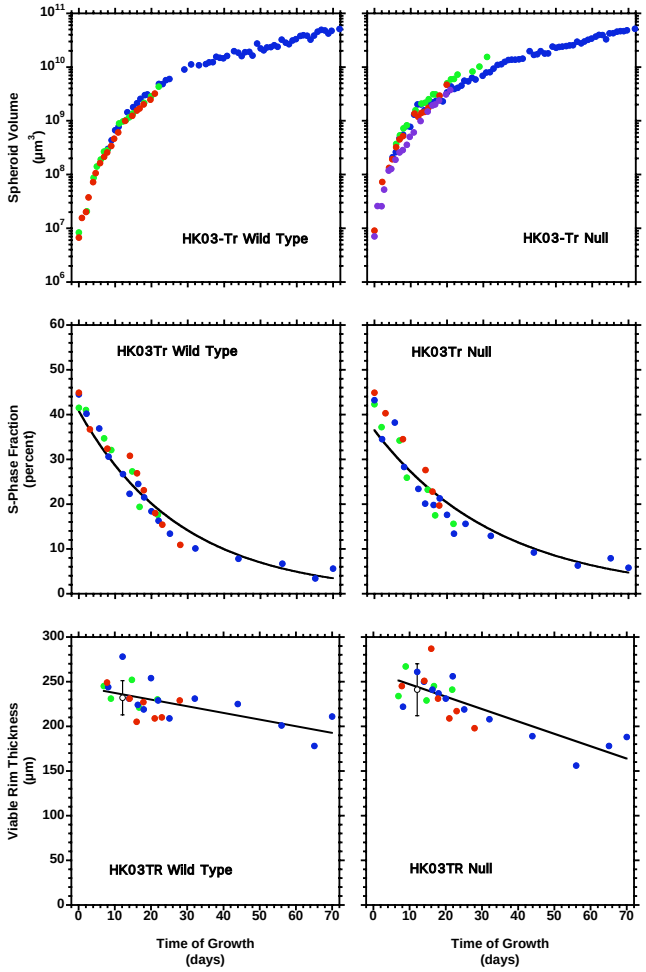
- Enormous complexity and heterogeneity both within and between tumors
- Non-reproducibility of even the best rodent tumor model systems
- Poor understanding of extent and control of transient variations: basically chaos
- Inability to control experimental parameters
- Inability to perform mechanistic experiments on humans
- Therefore, advances in basic understanding of tumor biology (and progress in therapy?) require *in vitro* experimental models of tumor

In Vitro Experimental Tumor Models

- Most basic: monolayer or suspension cell cultures
  - Useful for very basic studies
  - A very poor model of a 3-D tissue
  - Do not mimic any aspect of the tumor microenvironment
- Several different 3-D *in vitro* models have been developed
  - Cells embedded in external matrix material
  - Bioreactors: cells within artificial capillary structure
  - 'Sandwich' culture: cells trapped between two plates
  - Multicell layers: 3-D layers of cells on a membrane
  - *Ex vivo* explants of tumor pieces
  - Multicellular aggregates: spherical 3-D cultures ('spheroids')

Multicellular Tumor Spheroids





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Similarities: Spheroids and Tumors

- 3-D, tissue-like structure
  - Cell-cell contacts
  - Extracellular matrix
  - Microenvironment develops spontaneously
- Heterogeneous microenvironment
  - Gradients in extracellular biochemistry
  - Gradients in cellular physiology
  - Gradients in cellular metabolism
  - Gradients in gene/protein expression
- Therapy resistance
  - Radiation (ionizing, UV, microwave)
  - Many forms of chemotherapy
  - Hyperthermia
  - Photodynamic therapy
  - Biologicals (antibodies, liposomes, nanoparticles)

Advantages: Spheroids *vs* Tumors

- Highly reproducible
  - Very small inter-spheroid variability
  - Excellent long-term ‘stability’ (decades)
- Symmetrical
  - Gradients are radially distributed
  - Various gradients are tightly correlated
  - Enables some unique experimental manipulations
  - Ideal for mathematical modeling
- Experimental control
  - External environment controlled
  - Reproducible manipulation of experimental conditions
  - Easy to manipulate individual spheroids
  - High ‘data density’

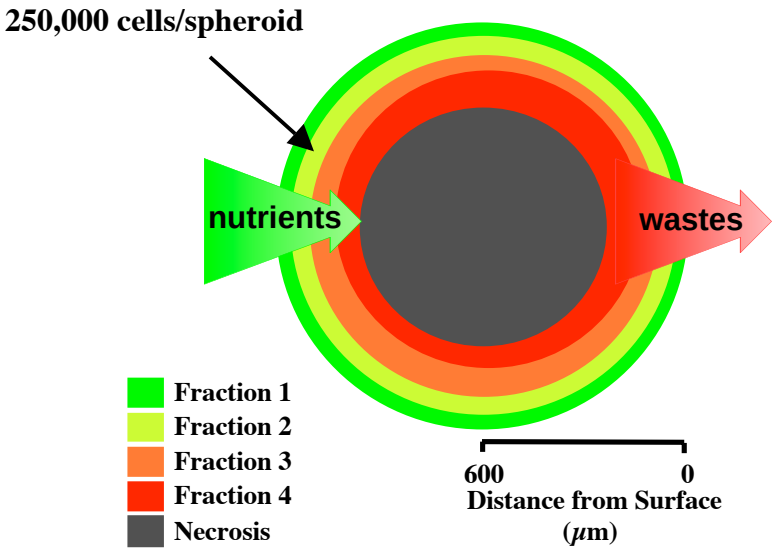
Research applications of spheroids

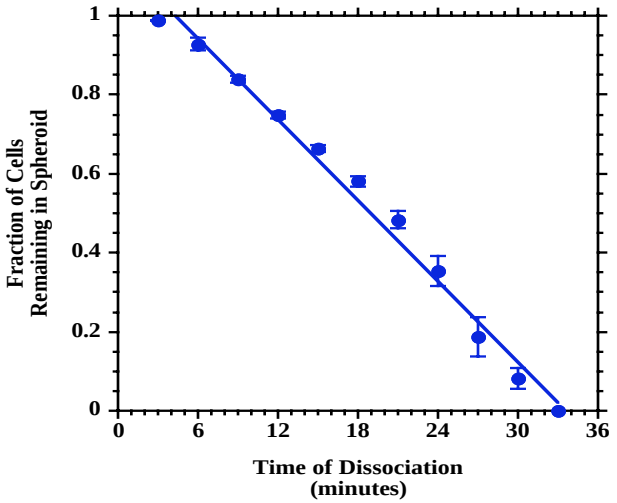
- Therapy testing and mechanistic studies
- Basic tumor biology
  - Cell cycle regulation
  - Metabolic regulation
  - Cellular physiology
  - Cell-cell interactions
  - Regulation of gene/protein expression
  - Malignant progression
- Co-cultures
  - Tumor-normal cell mixtures
  - Angiogenesis models
- Non-cancer applications
  - Artificial organ research
  - Drug production
  - Normal tissue models

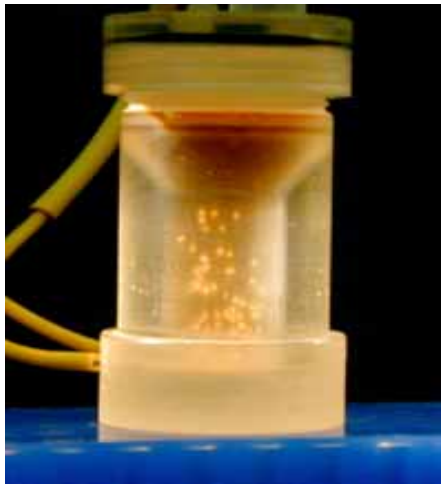
Example: Cell Cycle Regulation

- Despite common (mis)conception that malignant cells have escaped growth control, majority of tumor cells in a solid tumor are not proliferating
- Common (mis)dogma is that cell cycle arrest in tumors is due to lack of nutrients, specifically oxygen
- Although recent imaging and molecular techniques have documented spatial distribution of proliferation in rodent and human tumors, controlled manipulation and mechanistic experiments are not possible
- Actual molecular mechanism of cell cycle arrest in tumors is currently unknown
- Spheroids are a good *in vitro* model to perform mechanistic studies on this question

Multicellular Tumor Spheroids







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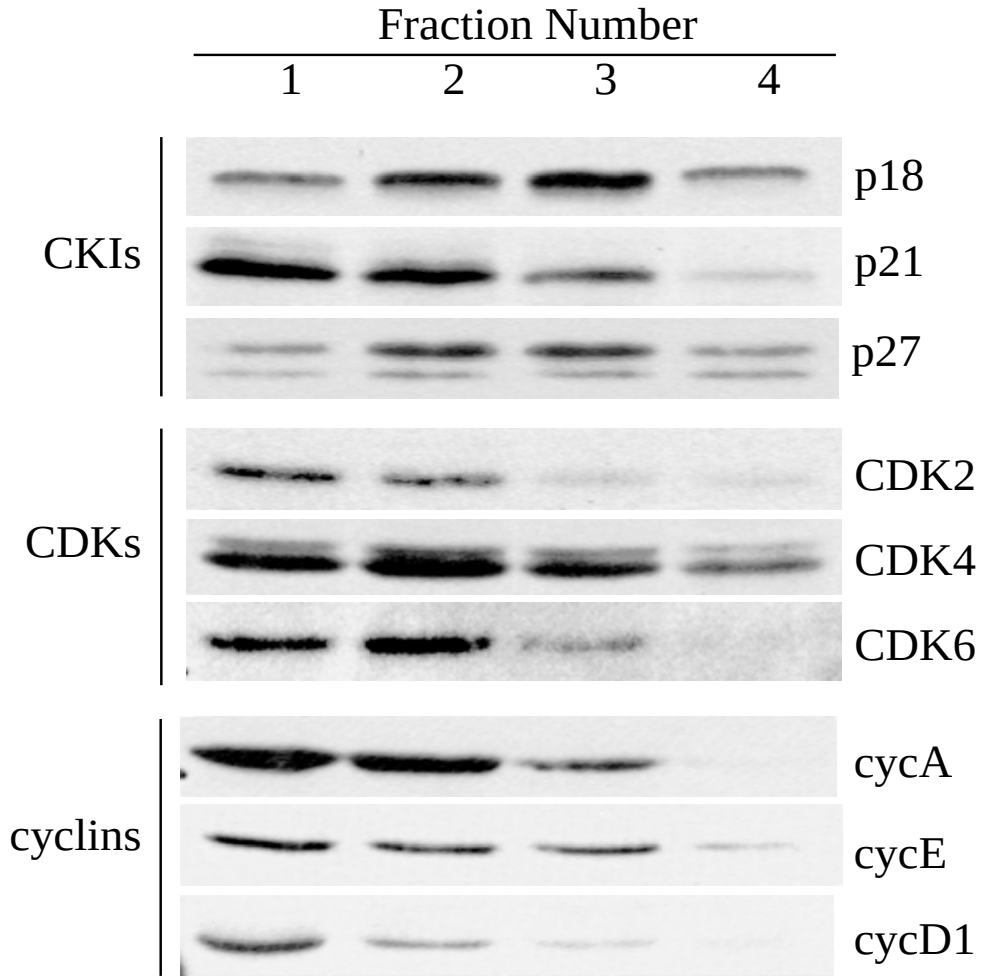
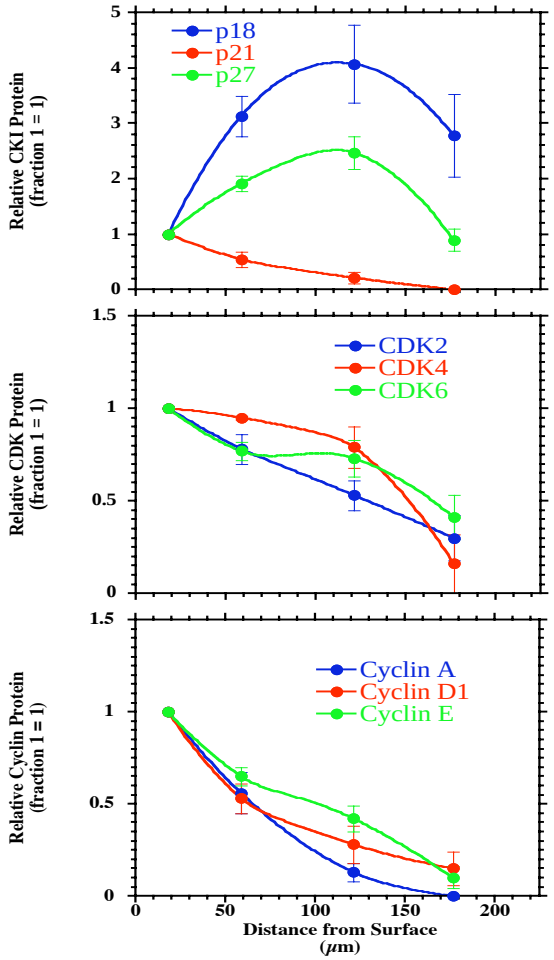
Cell Cycle Proteins in Spheroids





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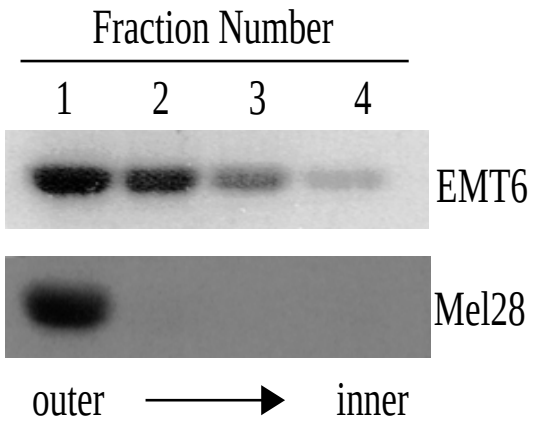
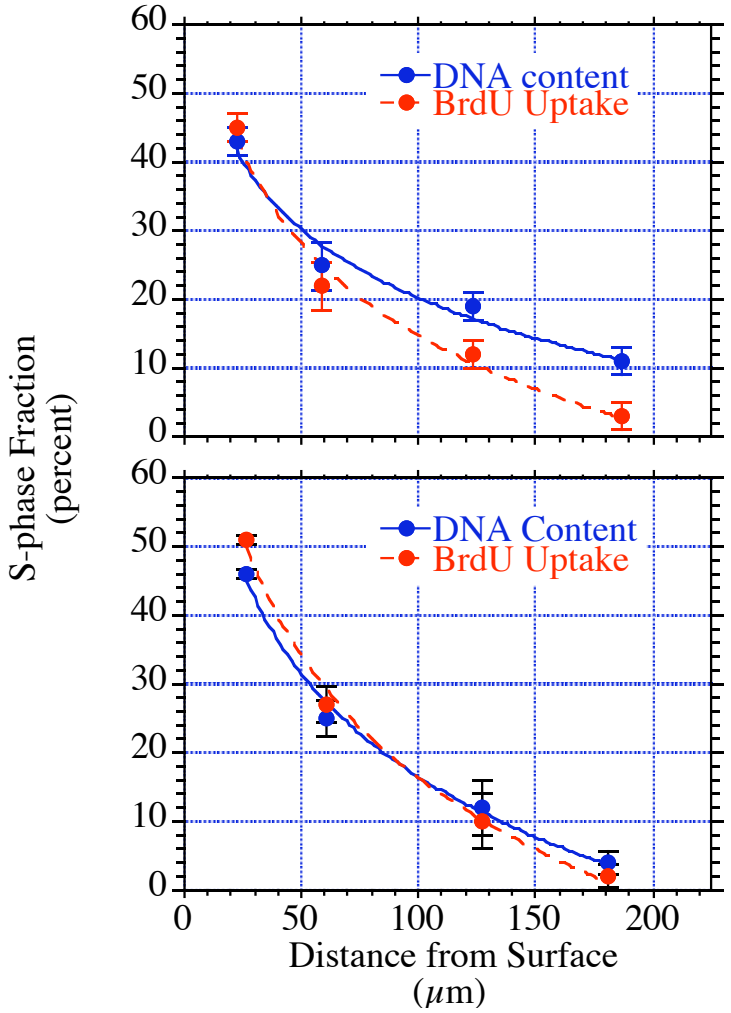
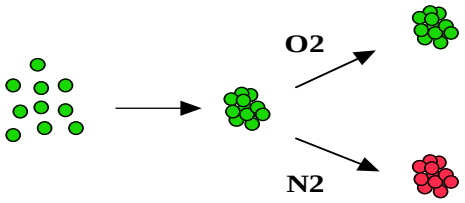
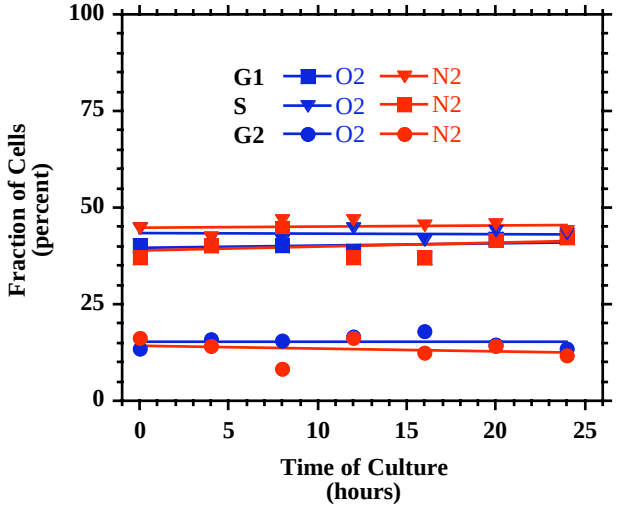
G1- Versus S-phase Arrest

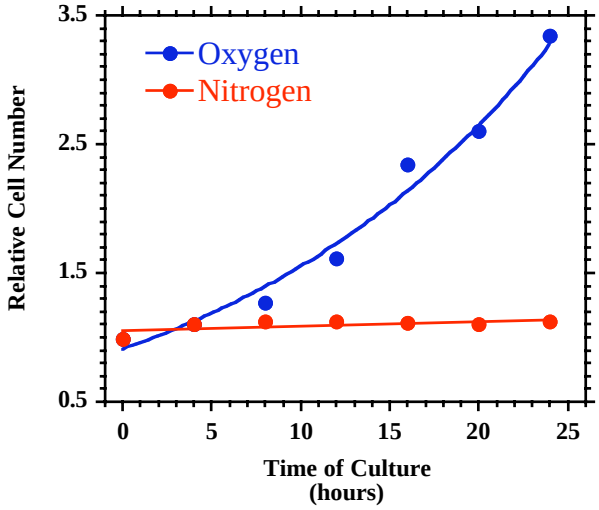


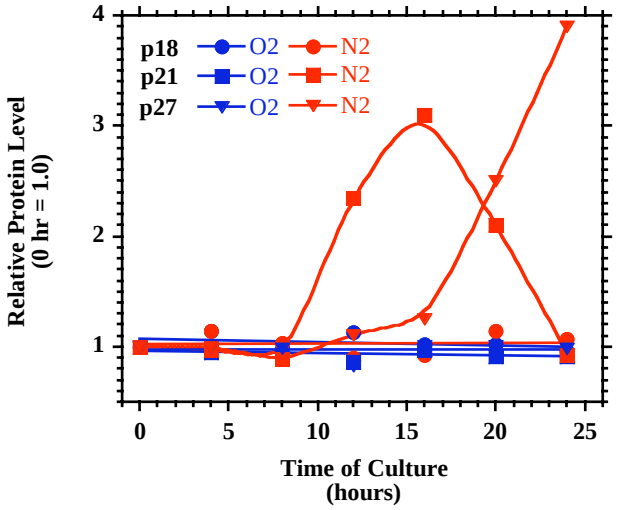


Cell Cycle Arrest After Acute Oxygen Deprivation









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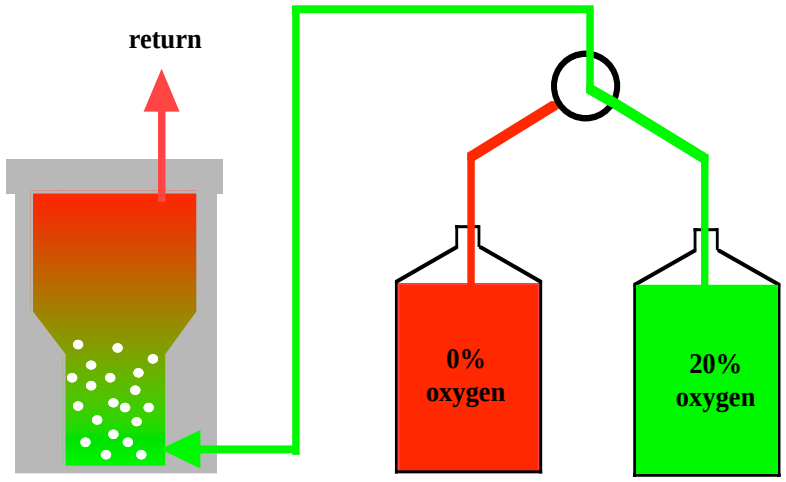
Regulation of Proliferation in Spheroids

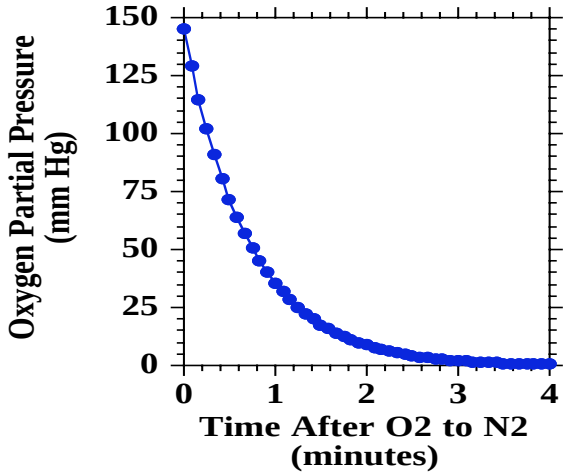
- Initial arrest is an *active* process regulated by a cyclin/CDK mechanism
  - Little change in CDKs, loss of cyclin D1
  - Upregulation of p18 and p27, loss of p21
  - CKI binding to and inhibition of CDK activity
  - Bypassing initial G1-arrest allows S-phase arrest
- Interior arrested cells continue to undergo alterations in cell cycle regulatory machinery
  - Loss of all regulatory molecules: CDKs, cyclins, CKIs
  - May explain prolonged recovery lag time: unable to resume without rebuilding?
- Inducers of initial arrest currently unknown
  - Several CKIs, up- and down-regulated: multiple signals?
  - Initiated relatively close to surface (~50 μm)
  - Unlikely to be related to oxygen deprivation
  - Growth factor or inhibitor? Pressure sensing?

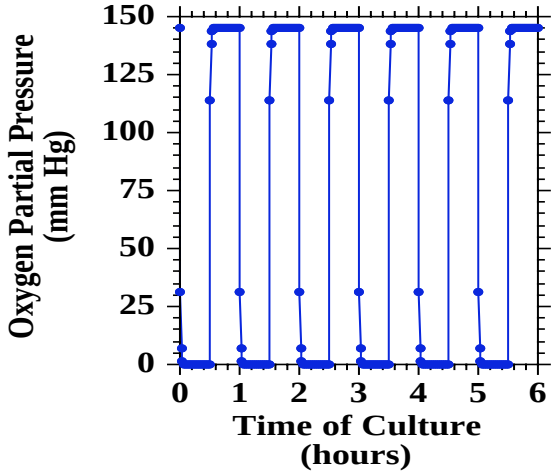
Limitations to Current Spheroid Model Systems

- Only mimics chronic nutrient deprivation
- Difficult for *in situ* assay of microenvironmental gradients (microelectrodes, histology)
- Separation of cells from different locations involves relatively long enzymatic treatment (complicates gene and protein expression data)
- Only applicable to adherent cells and those that proliferate in aggregate culture
- Difficult to use for controlled, reproducible experiments with co-cultures

Transient Deprivation System for Spheroids







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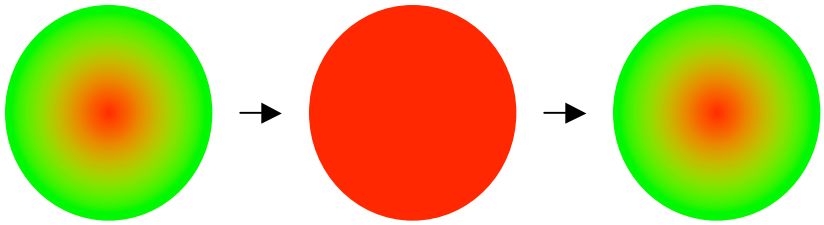
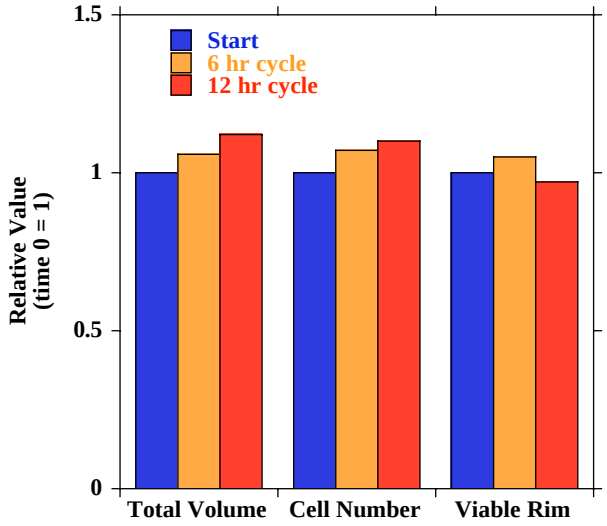
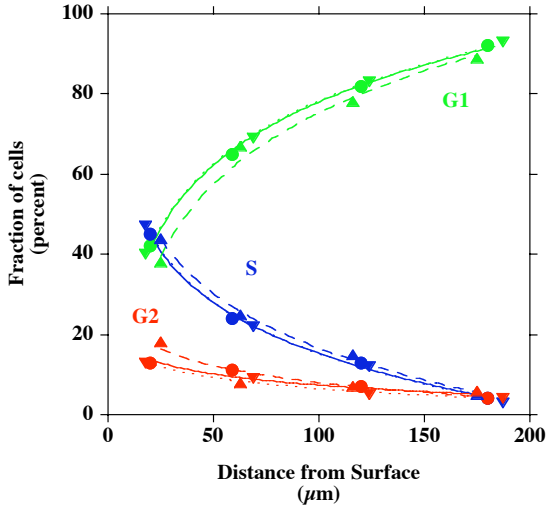
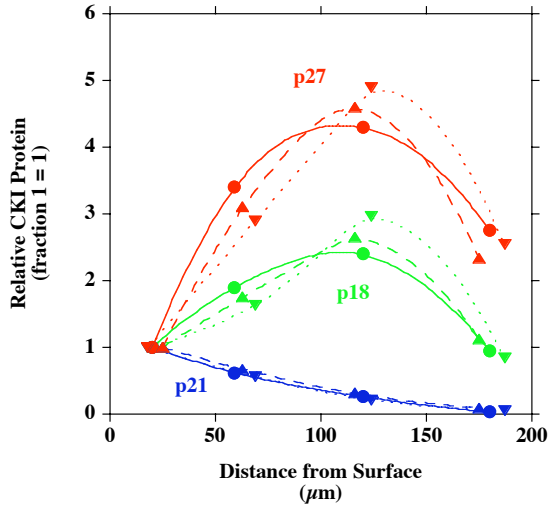
Effects of Transient Oxygen Deprivation



30 minutes oxygen | 30 minutes nitrogen | 30 minutes oxygen







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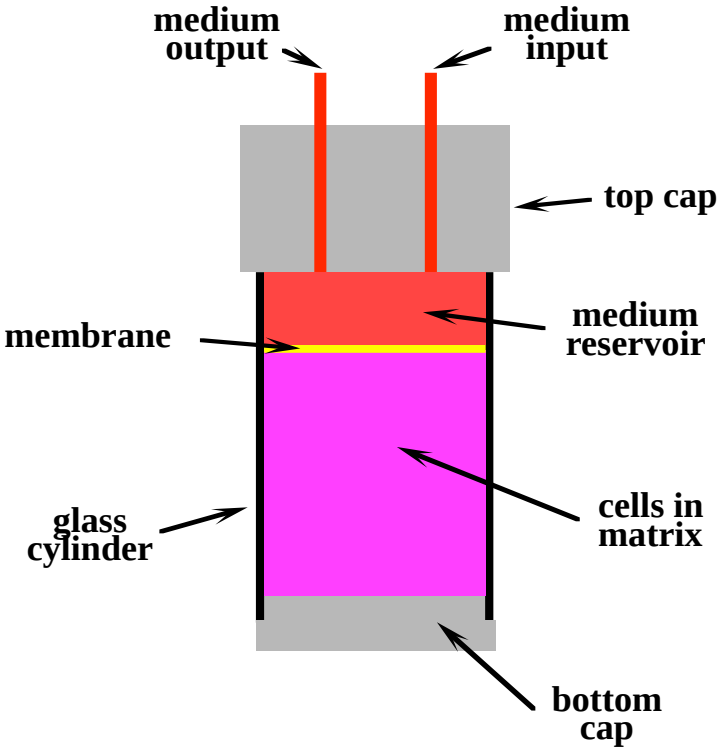
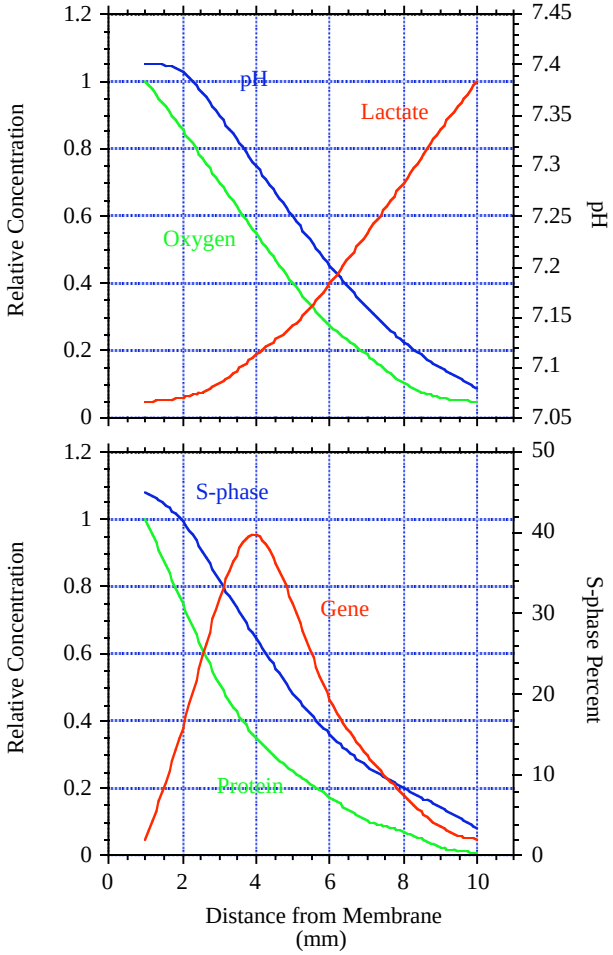
Transient Nutrient Deprivation in Spheroids

- New culture system developed and validated for transient deprivation experiments
  - Compact, portable culture chamber
  - Ability to rapidly alter nutrient conditions
  - Imposes external transient supply on pre-existing chronic gradients: more like tumor *in vivo*
- Preliminary experiments show essentially no effect of cyclic oxygen supply for up to 12 hours
  - No change in spheroid growth rate or cell number
  - No increase in central necrosis
  - No alteration in cell cycle or CKI induction
- Preliminary experiments show remarkable resistance to nutrient deprivation
  - Complete nutrient deprivation causes total loss of ATP and extremely acidic intracellular pH
  - Complete recovery of normal cellular energetics after nutrient restoration

New *In Vitro* Model of Tumor Microenvironment





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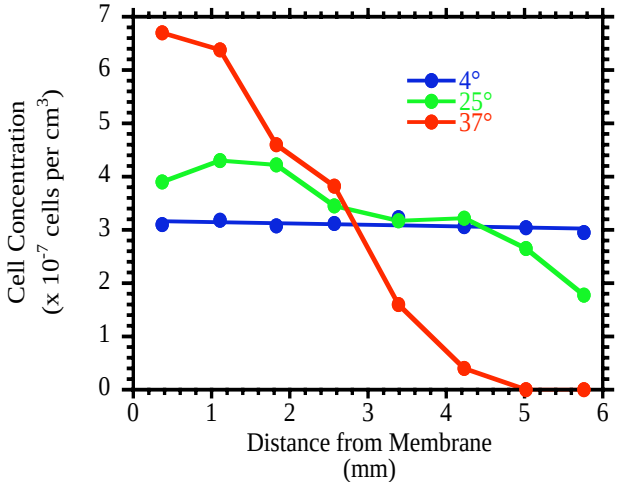
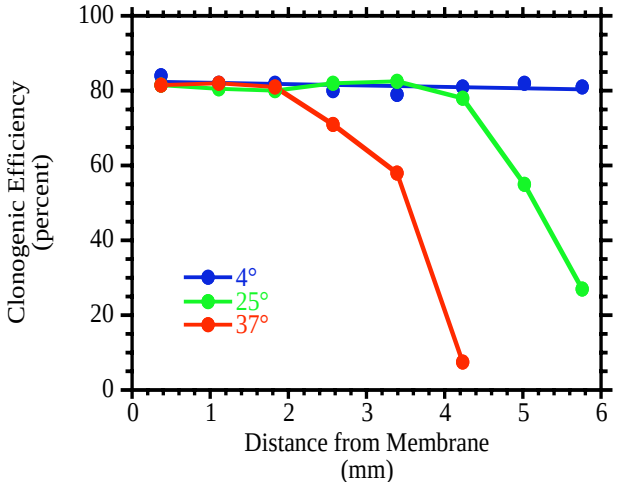
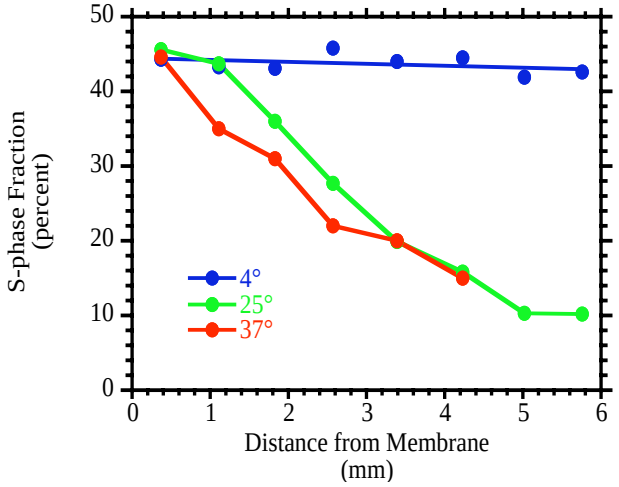
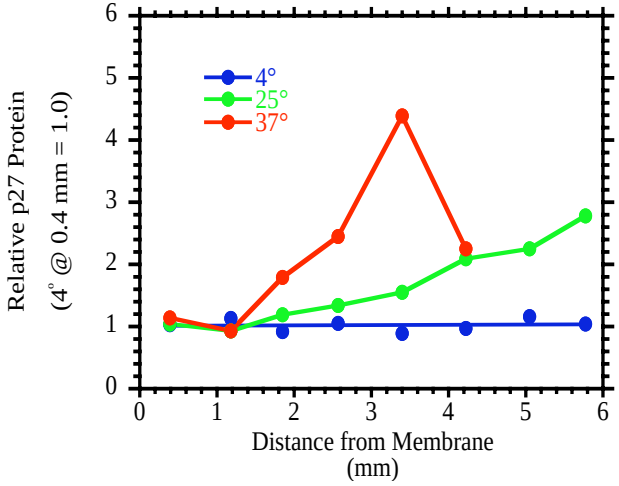
Preliminary Data with 1st Generation System









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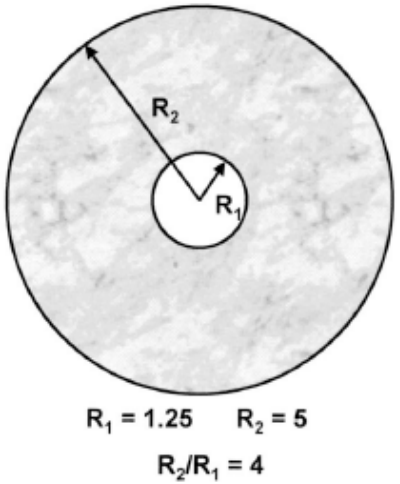
Current State of New Model System

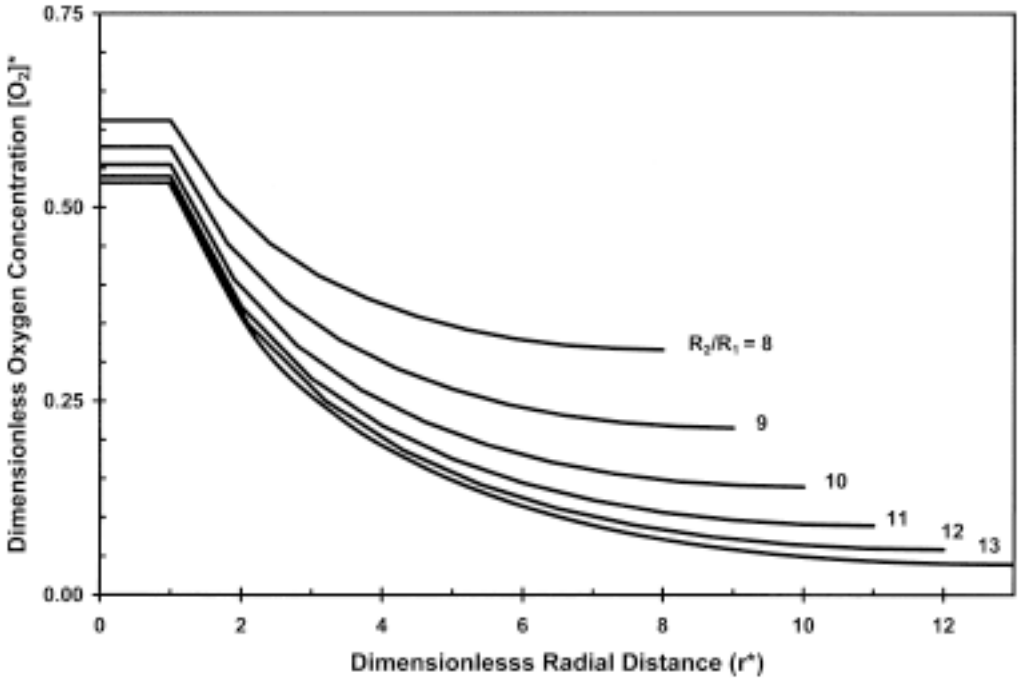
- Demonstration of feasibility of design
  - Spatial correlation of microenvironment and biology
  - Potential for real-time, in situ measurement by NMR
  - Allows rapid isolation of cells from different regions
  - Experimental control over many parameters
- Produces physiological gradients similar to those seen in spheroids and tumors
  - Cell proliferation and cell cycle distribution
  - Cell death
  - Induction of CKIs
- 1st generation system has problems
  - Difficult and non-reproducible separation of cells from different regions, still requires matrix digestion
  - No control over internal supply conditions
  - Relatively low cell number to get extended gradients

Theoretical Modeling of Tumors

- Overwhelming majority of literature based on mathematical models of tumor growth and development (~1200 papers since 1970)
- Interestingly, spheroid growth data very often used to 'test' models
- Limited development in other areas
  - Interactions with immune system
  - Regulation of cellular metabolism
  - Extracellular biochemical environment
  - Cellular invasion
  - Therapy response (radiation, chemo)
  - Protein regulatory networks
- Recent focus on developing biologically-based models of tumor growth and malignant progression

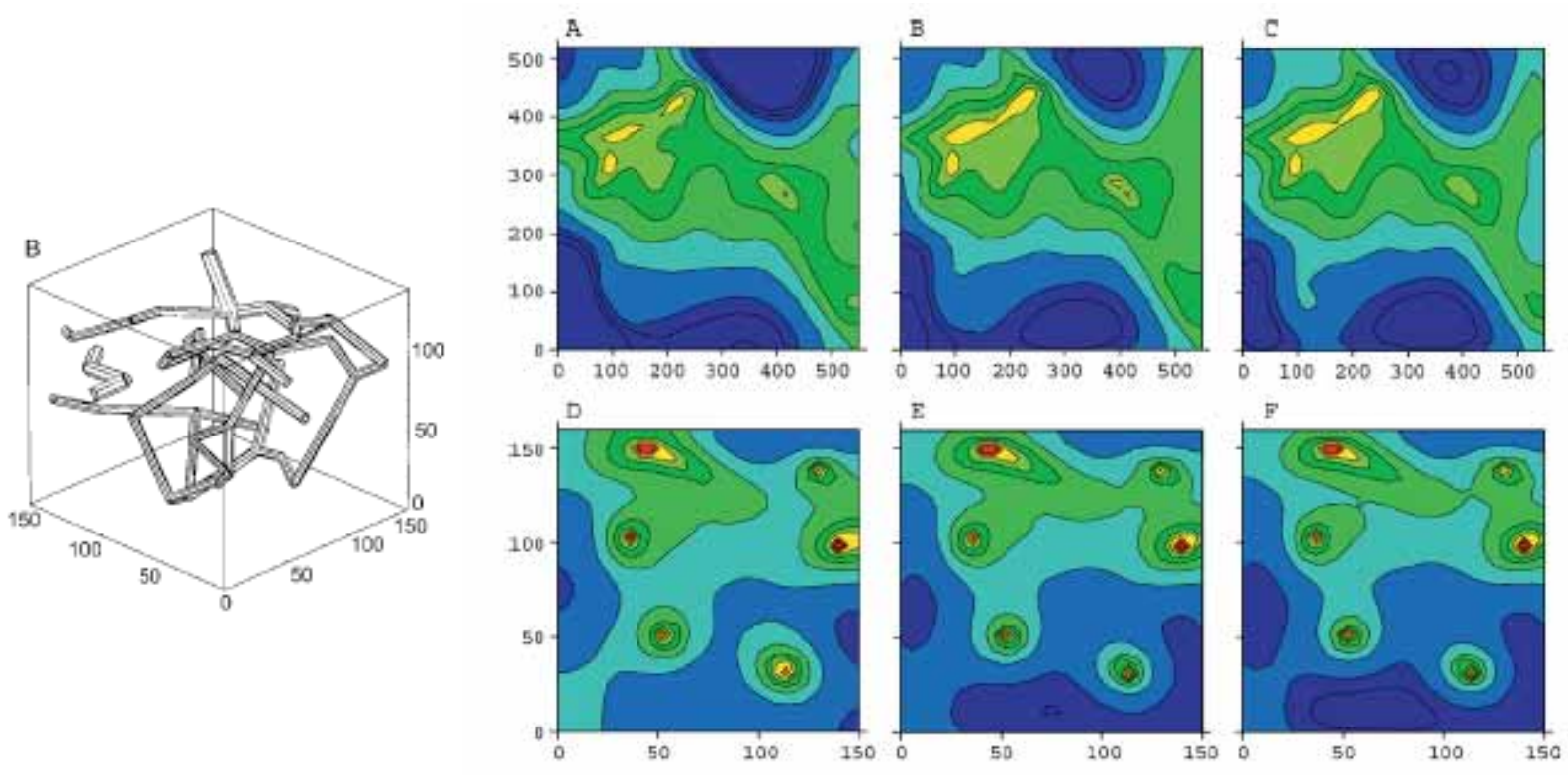
Modeling Hypoxia in Tumors





Kirkpatrick et al., *Radiat. Res.* 159: 336, 2003

Modeling Hypoxia in Tumors



Secomb et al., *Annal. Biomed. Engineer.* 32: 1519, 2004

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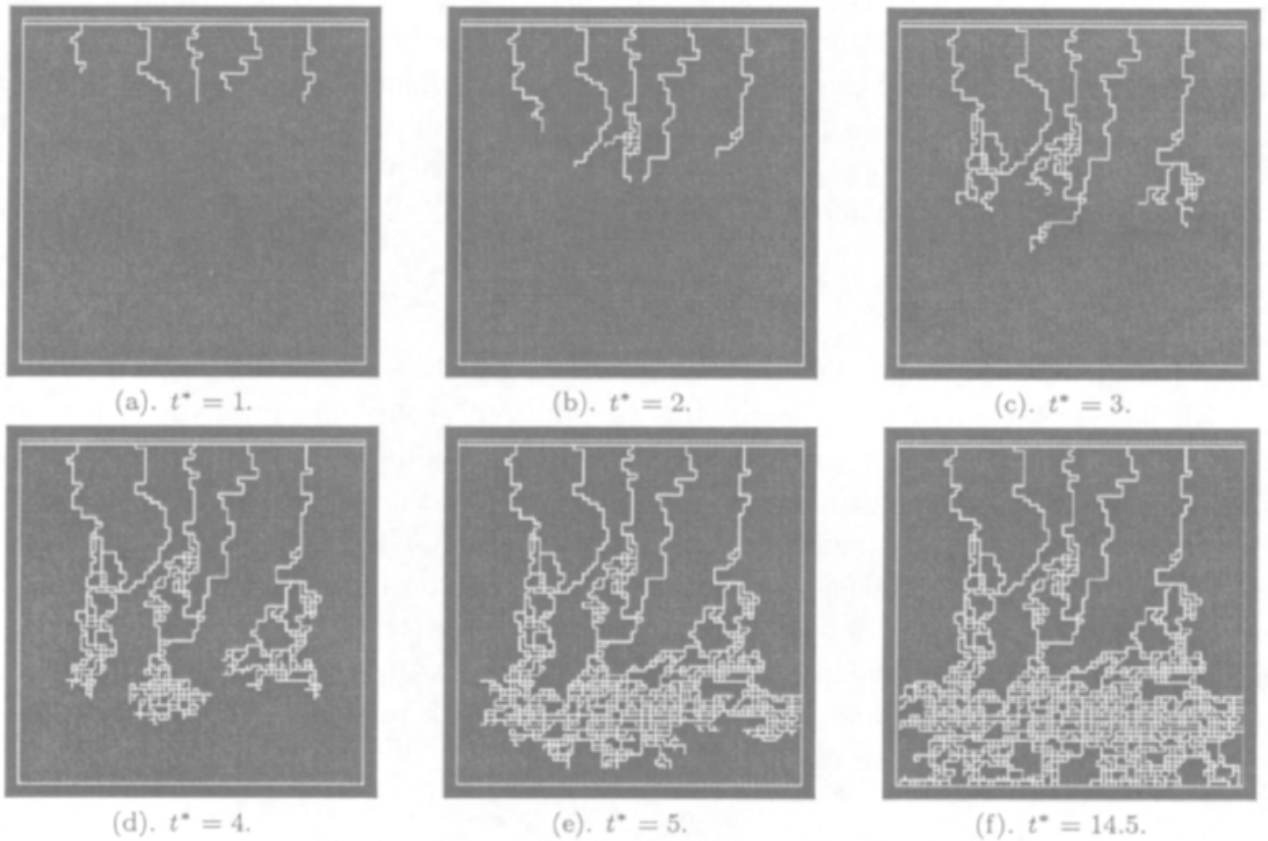
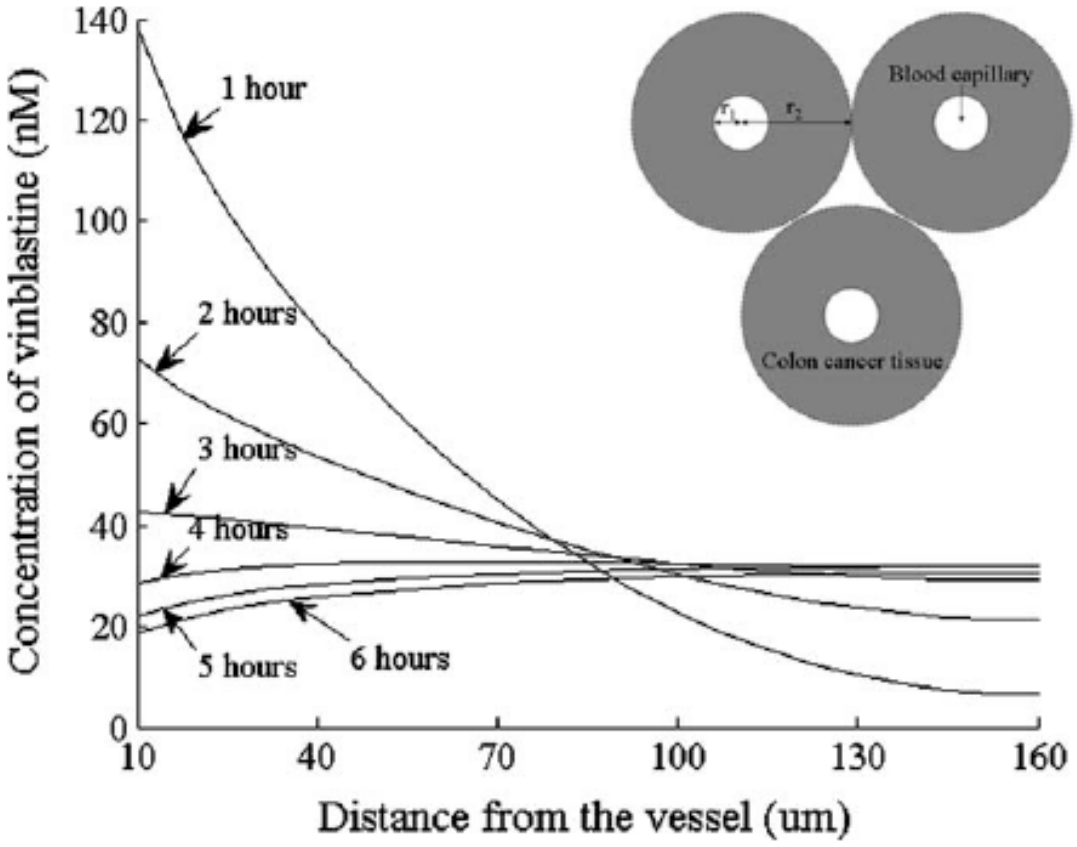
Modeling Angiogenesis in Tumors



Stephanou et al., *Math. Comput. Model.* 41: 1137, 2005

Penetration of Chemotherapy Agent



Modak et al., *Eur. J. Cancer.* 42: 4204, 2006

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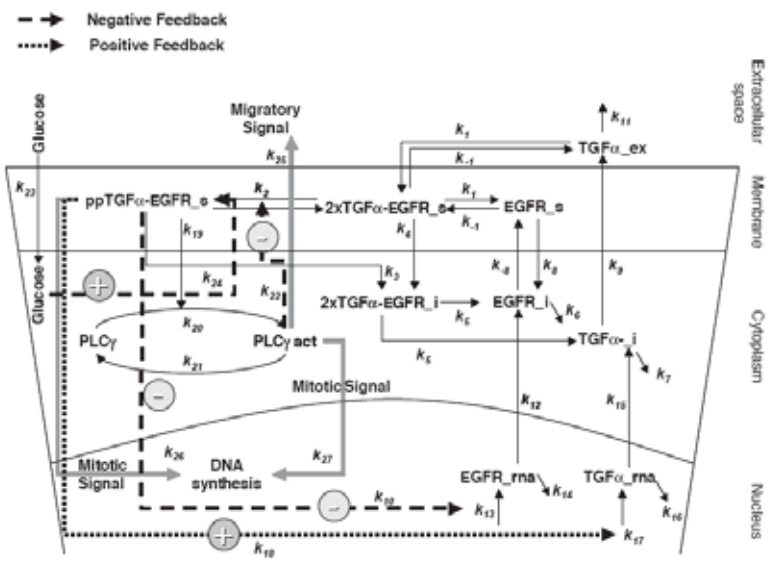
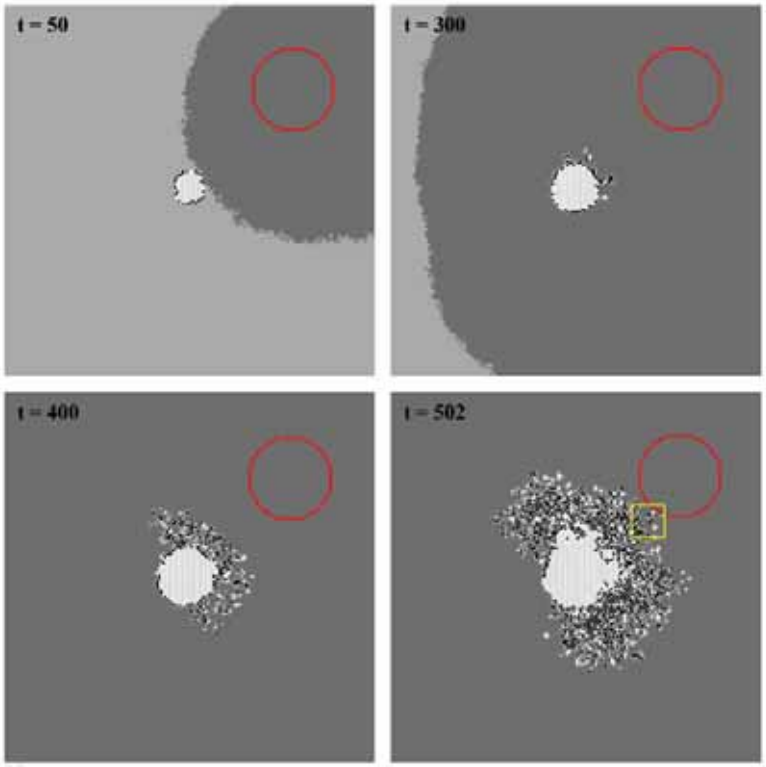
Protein Network Model of Tumor Cell Invasion

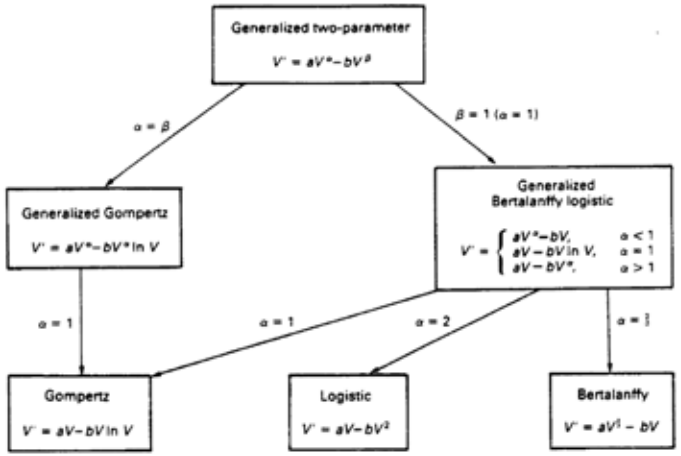




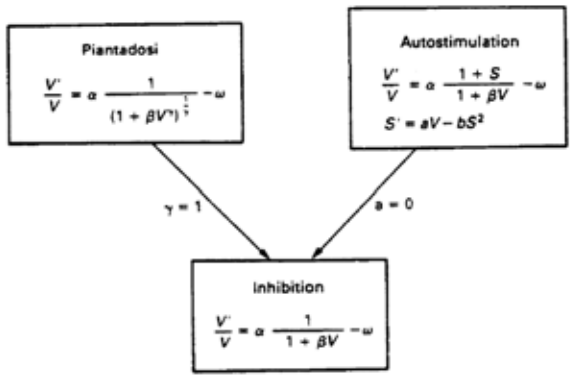
Athale et al., *J. Theor. Biol.* 233: 469, 2004

Nested Deterministic Models of Tumor Growth

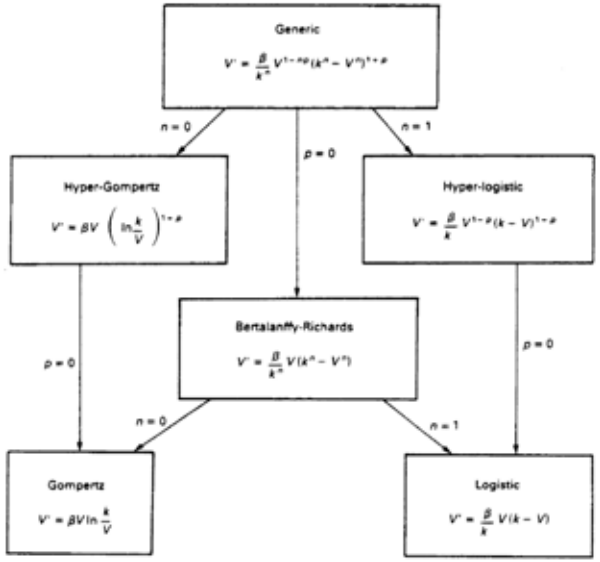
Two-parameter Models



Functional Models

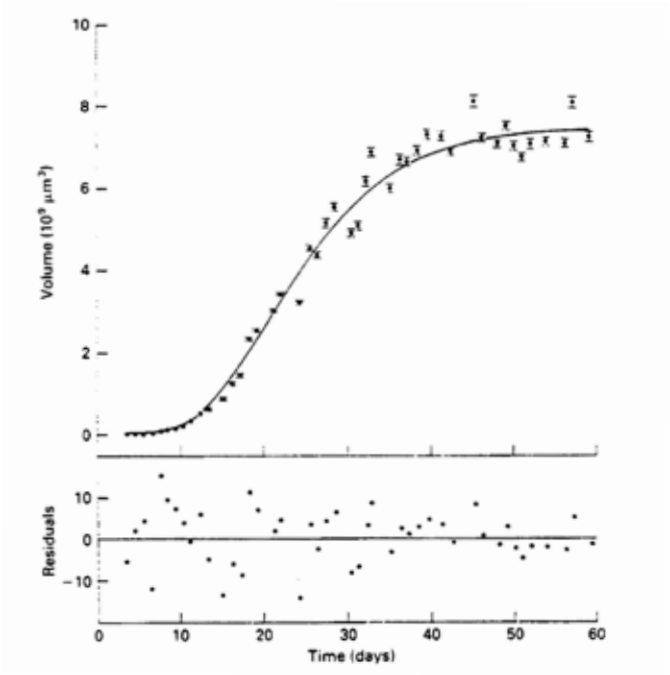


Generic Models



Marusic et al., *Cell Prolif.* 27: 73, 1994

Fits of 15 Models to 15 Independent Data Sets



Data No. of free parameters	G* 3	GG 4	L 3	B 3	GBL† 4	GTP 5	HG 4	HL 4	Ge 5	AS 6	P 5	I 4	SS 4	LFS 8	Pol.	
1	1.448	1.391	88.838	50.880	1.391	1.391	1.409	2.324	1.376	1.183	1.338	5.372	2.059	2.084	55.2	(7)
2	0.875	0.876	22.005	13.218	0.876	0.876	0.876	1.316	0.873	0.912	0.872	2.903	1.331	0.884	318.3	(6)
3	1.048	1.045	8.045	5.903	1.045	1.045	1.047	1.303	1.030	0.873	1.007	1.418	1.037	1.028	1803.4	(7)
4	1.150	1.049	18.221	10.185	1.048	0.967	1.095	1.048	1.048	0.982	1.050	3.624	1.863	0.978	465.9	(5)
5	1.081	1.002	4.302	4.033	1.003	1.002	1.046	1.162	0.969	0.904	0.945	1.038	0.976	0.806	1856.0	(6)
6	1.038	0.987	12.653	7.568	0.987	0.972	1.011	1.069	1.011	0.882	0.987	2.654	1.410	0.968	1731.1	(7)
7	1.379	1.261	2.664	2.977	1.265	1.261	1.313	1.375	1.252	0.978	1.162	1.162	1.109	1.200	1559.2	(6)
8	1.067	1.039	18.806	9.757	1.039	1.039	1.054	1.141	0.972	0.813	1.022	1.574	1.226	0.752	168.9	(7)
9	1.287	1.175	26.653	10.663	1.175	1.174	1.185	1.371	1.185	1.284	1.176	2.780	1.553	1.287	446.8	(6)
10	1.033	1.031	10.303	7.079	1.031	1.031	1.030	1.214	1.030	1.043	1.031	1.658	1.288	1.179	2359.4	(7)
11	1.212	1.158	6.031	5.323	1.159	1.158	1.192	1.335	1.111	0.992	1.032	1.067	1.023	1.163	1291.6	(7)
12	1.414	1.158	16.098	12.016	1.158	1.158	1.122	1.334	1.122	0.971	1.158	1.861	1.342	1.437	145.4	(6)
13	1.157	1.011	4.701	4.780	1.011	1.011	1.039	0.996	0.996	1.011	1.011	1.178	1.098	0.928	731.8	(8)
14	1.124	1.113	10.658	8.889	1.113	1.113	1.115	1.403	1.113	0.968	1.107	2.234	1.324	1.067	1168.5	(8)
15	1.056	1.045	15.630	12.645	1.045	1.044	1.041	1.257	1.041	1.088	1.045	2.591	1.531	1.262	1087.2	(9)

Marusic et al., *Cell Prolif.* 27: 73, 1994

Fits of 15 Models to 15 Independent Data Sets

Doubling Time

	Time (h)						
Data	Exp	G	AS	P_1	P_2	SS	LFS
1	12	11.3	15.8	11.7	19.6	14.9	> 1 year
2	13	8.7	13.1	9.3	14.4	14.5	13.1
3	9.5	13.7	17.1	18.8	22.4	20.6	14.6
4	17	7.6	12.2	4.4	13.1	13.8	4.2
5	20	11.6	19.9	21.0	26.1	18.9	7.6
6	10	10.7	14.3	4.4	15.4	15.2	2.3
7	13	10.7	36.3	23.5	28.6	15.2	13.2
8	14	14.3	11.5	16.1	25.8	17.8	5.3
9	15	13.5	18.7	6.9	20.2	18.7	> 1 year
10	18	20.3	19.4	9.6	27.7	29.2	12.2
11	12	9.2	17.4	19.2	22.3	15.0	7.4
12	18	8.9	8.9	4.0	17.3	12.1	> 1 year
13	15	6.1	6.7	2.4	17.4	10.8	> 1 year
14	11	13.3	18.8	12.4	20.6	19.6	6.9
15	10	9.4	14.6	2.8	16.0	15.1	1.8

Thickness of Viable Cell Rim

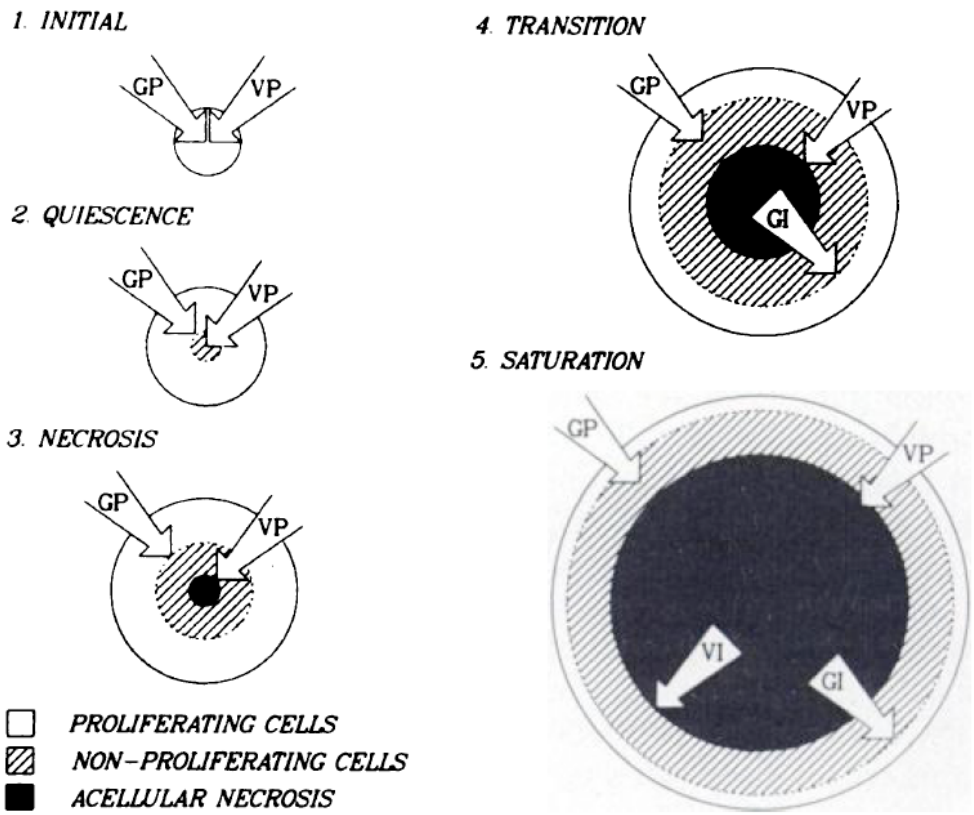
Data	Exp	AS	P	P_r	SS	LFS
1	118	67	77	97	85	777
2	257	71	98	217	129	109
3	251	135	87	90	128	79
4	213	95	125	499	129	46
5	238	95	105	77	145	45
6	180	92	118	416	115	21
7	98	176	72	32	96	50
8	85	30	70	74	79	18
9	198	89	103	292	97	1007
10	225	47	123	515	157	69
11	139	99	86	39	107	39
12	176	89	116	196	84	739
13	104	31	138	239	97	571
14	228	108	91	210	129	48
15	135	109	152	500	124	17

Marusic et al., *Cell Prolif.* 27: 73, 1994

Deterministic Tumor Models

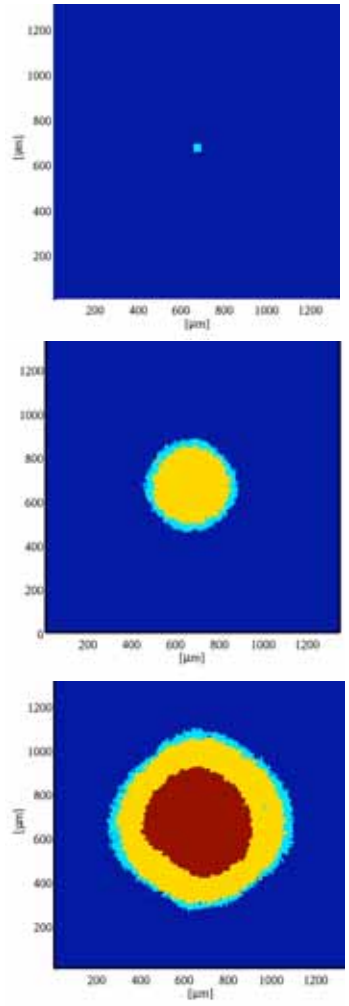
- Wide variety available and more being developed
- Most can do a good job of fitting basic tumor (spheroid) growth data
- Useful for graphing, comparing and extrapolating data
- Most do a poor job of predicting any biological parameters
- Not really useful for advancing our understanding of tumor biology
  - Generally not predictive
  - Many not directly connected to biology
  - Those that are have a very large number of parameters
  - Difficult to distinguish one from the other
- The future of this field is in biologically-based models

Conceptual Model of Spheroid Growth Regulation



Freyer & Sutherland, *Cancer Res.* 46: 3504, 1986

Multi-Scale Mathematical Tumor Model



- Starts with single cell on 3-D lattice
  - ‘Programmed’ with metabolic, gene regulation, cell cycle, volume growth rate, adhesion and cell death parameters
  - Assumes limited inward growth factor penetration and internal growth inhibitor production
  - Simulation runs until lattice is filled or spheroid saturates: nothing ‘fit’ or constrained
- Three scales considered
  - Cellular (lattice Monte Carlo)
  - Gene regulation (Boolean network)
  - Extracellular (reaction-diffusion equations)

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Final Conclusions

- Solid tumors are perhaps the most unique, complex, dynamic and chaotic biological system
- The tumor microenvironment is extremely heterogeneous, both spatially and temporally
- This microenvironmental complexity explains most therapy failures, as well as promotes the progression of malignancy itself
- Actual tumors *in vivo* are poorly suited to mechanistic experimentation
- Many 3-D *in vitro* experimental tumor models are available and important for advancing tumor biology
- Spheroids are an excellent tumor model system, but have limitations
- Theoretical modeling of tumors is in its infancy, but can contribute significantly in cancer research

Acknowledgements

- Spheroid projects
  - Dr. Karen LaRue
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  - Jeffery Dietrich
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  - James Coulter
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- Flow cytometry
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