Photonics Meets Biophysics and Biology: From Nanoimaging to Winning the War on Cancer

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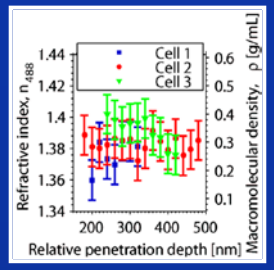
Backman Laboratory



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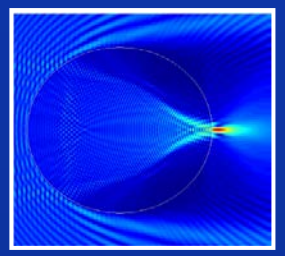
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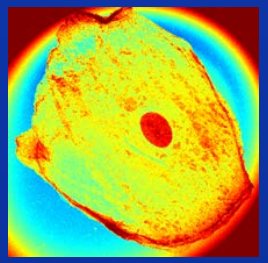
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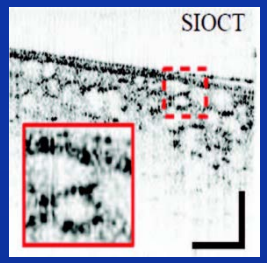
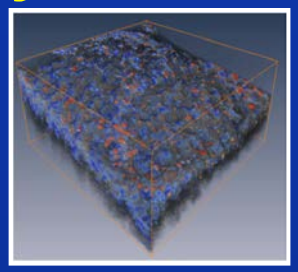


SI OCT/LSM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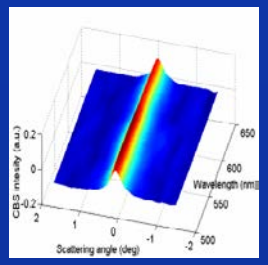
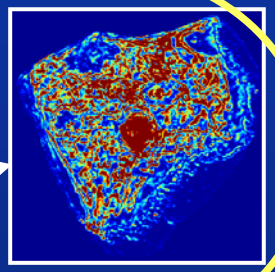


EBS


Clinical fiber-optics

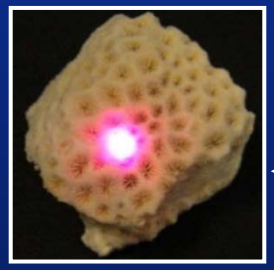

Nanocytology

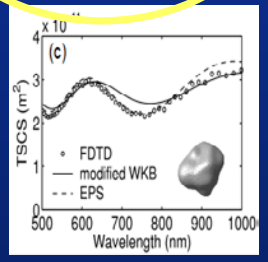
Technologies

Physics

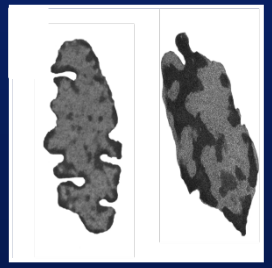
Biology

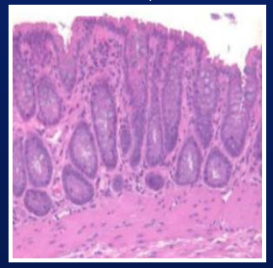
Medicine

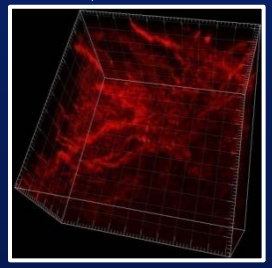

Coral reef bleaching

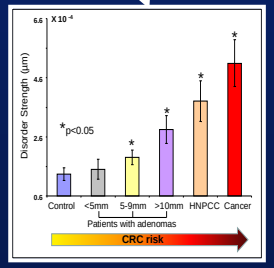


Light scattering










Metastatic sensor

Alterations in chromatin structure, microcirculation , and ECM in early carcinogenesis

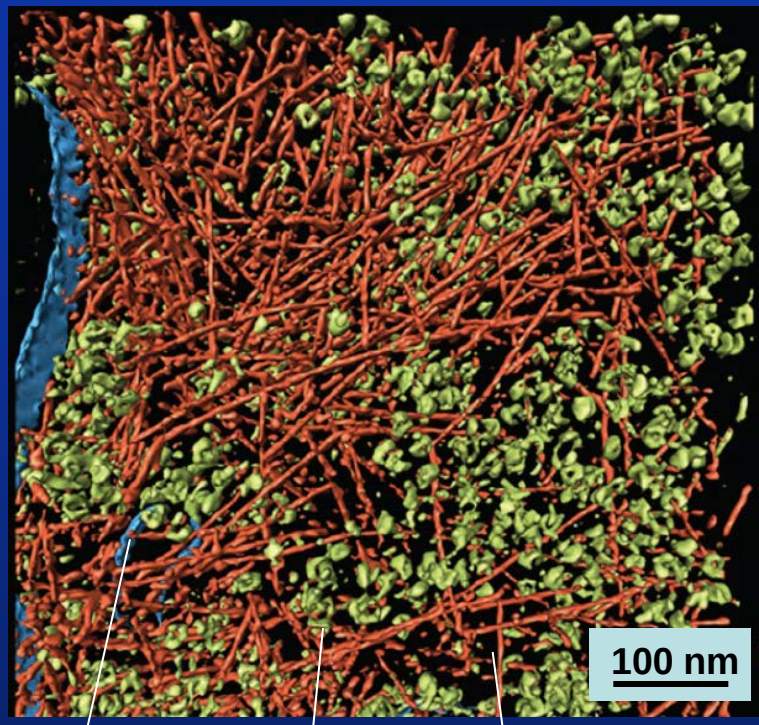
Screening /diagnosis of lung, colon, ovarian, pancreatic, esophageal, thyroid, and prostate cancers

Cell Organization: From Microscale to Nanoscale

- Cells have a highly complex organization:
  - Cellular level: ~ 10 μm
  - Organellar level: ~ 1 μm
  - Macromolecular level: < 0.1 μm
- Light microscopy does not resolve cell structures below ~200-500 nm.

Cell Nanoarchitecture

- Fundamental building blocks of a cell
- Cytoplasm: ribosomes, cytoskeleton, membranes
- Nucleus: nucleosomes, 30 nm chromatin fibers, higher-order chromatin structure



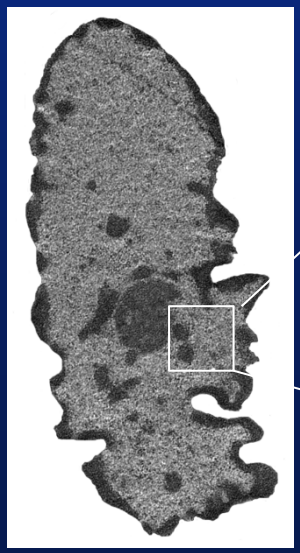
3D Reconstruction of cytoplasm, adapted from Medalia et al., 2002

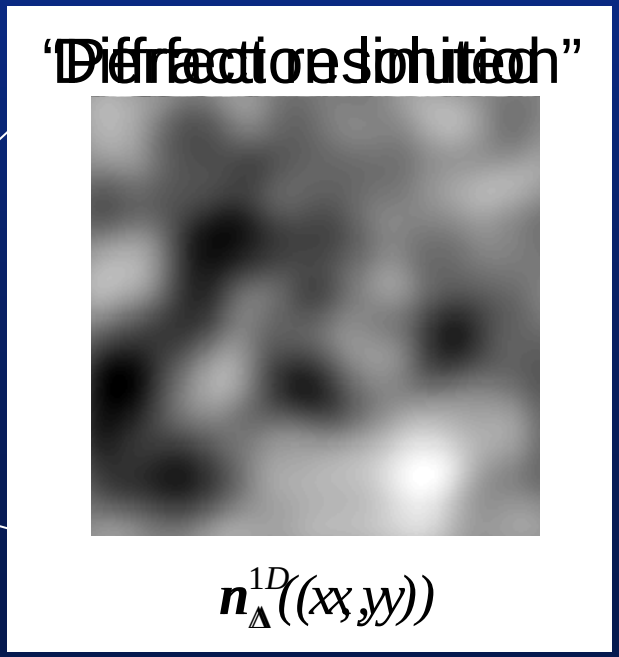
- Understanding cellular processes at the nanoscale has been stymied by the lack of practical means of analysis of cellular nanoscale architecture

Histology Cannot Measure Cellular Nanoarchitecture

- Conventional microscopic histology images cell micro-architecture but does not resolve cell structure below ~200-500 nm

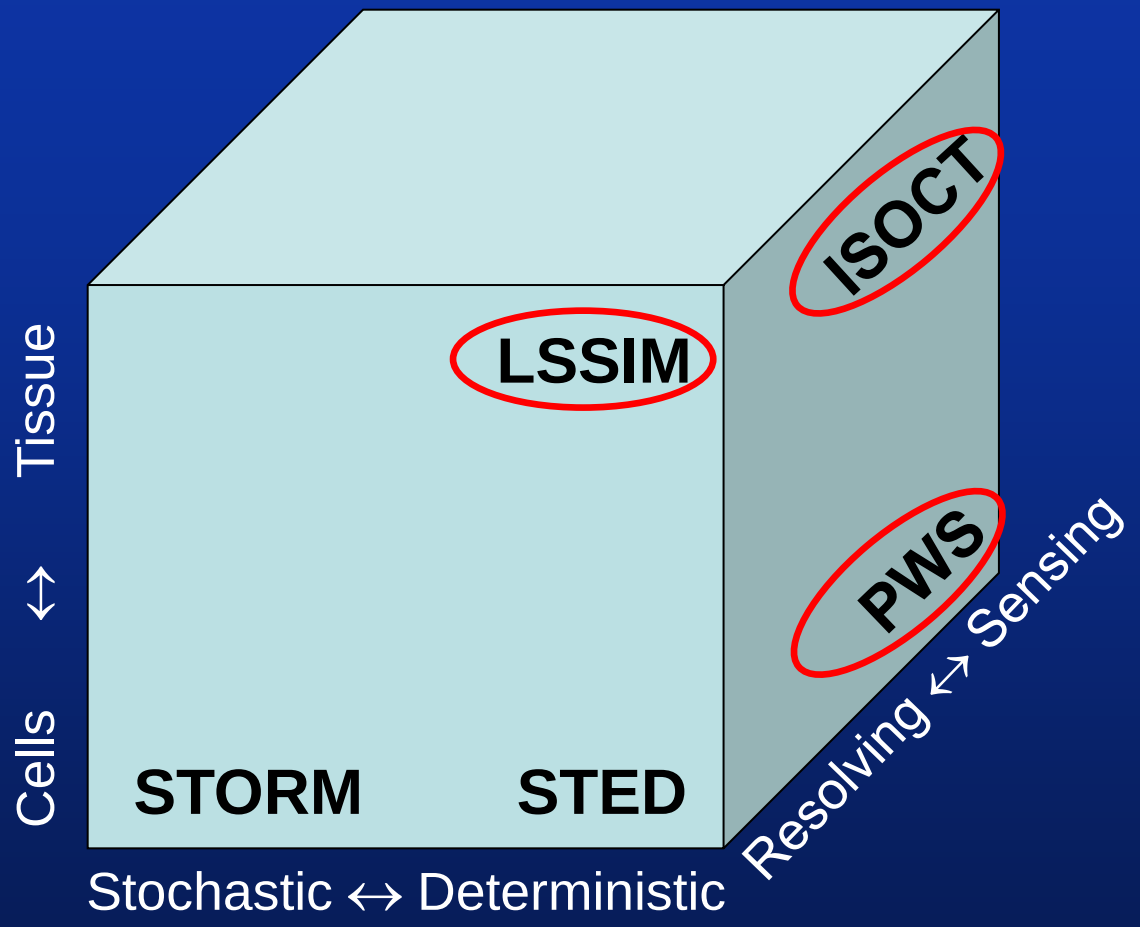
- How can we measure the statistical properties of spatial organization of macromolecular density with nanoscale sensitivity
  - Electron microscopy is too variable, cumbersome, and expensive
  - Optical techniques?



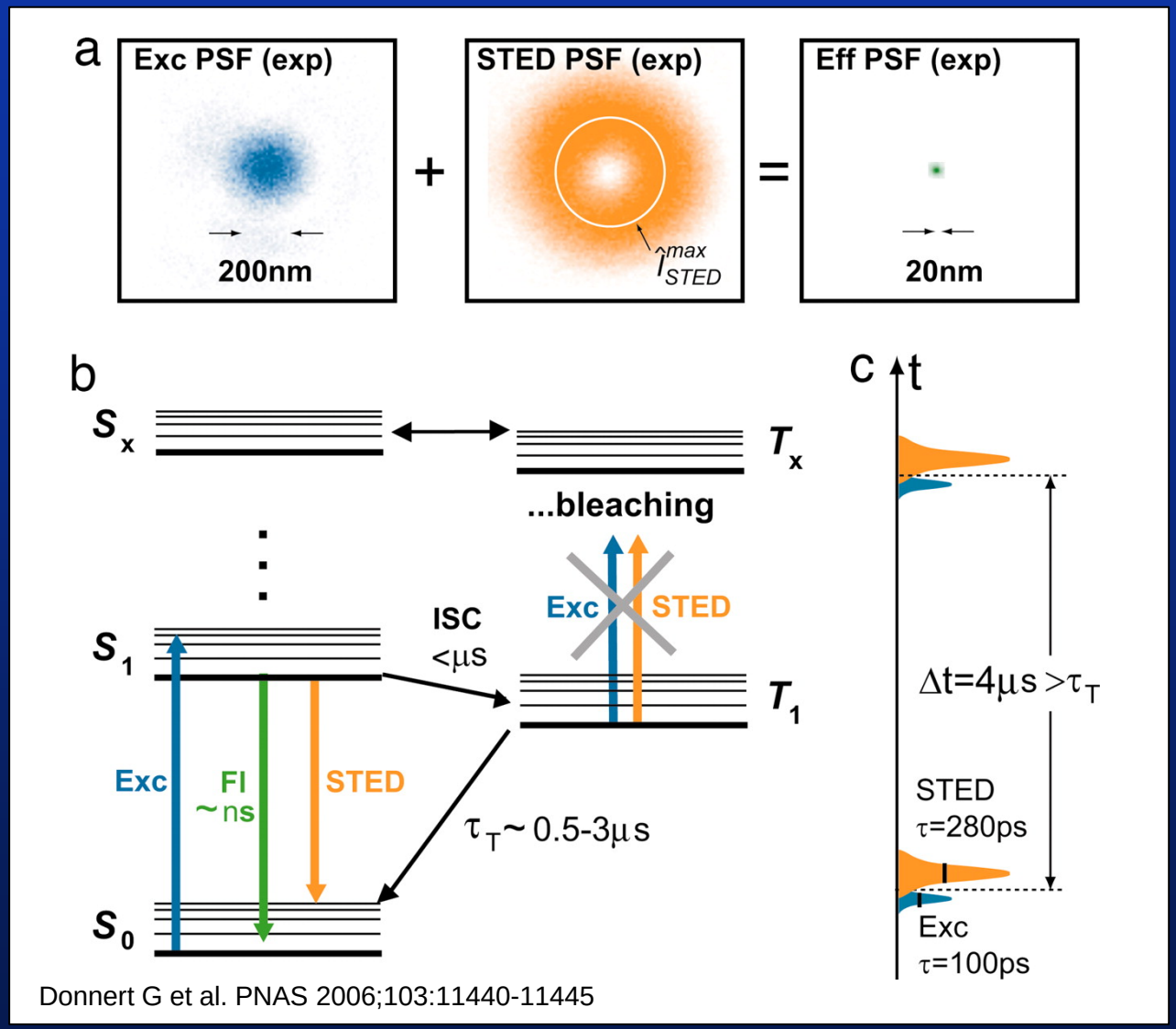


The ‘Universe’ of Subdiffractional Microscopy

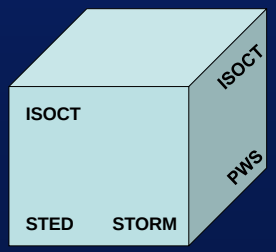
- STED – stimulated emission depletion microscopy
- STORM - stochastic optical reconstruction microscopy
- LSSIM – laser scanning structured illumination microscopy
- PWS – partial wave spectroscopic microscopy
- ISOCT – inverse spectroscopic optical coherence tomography



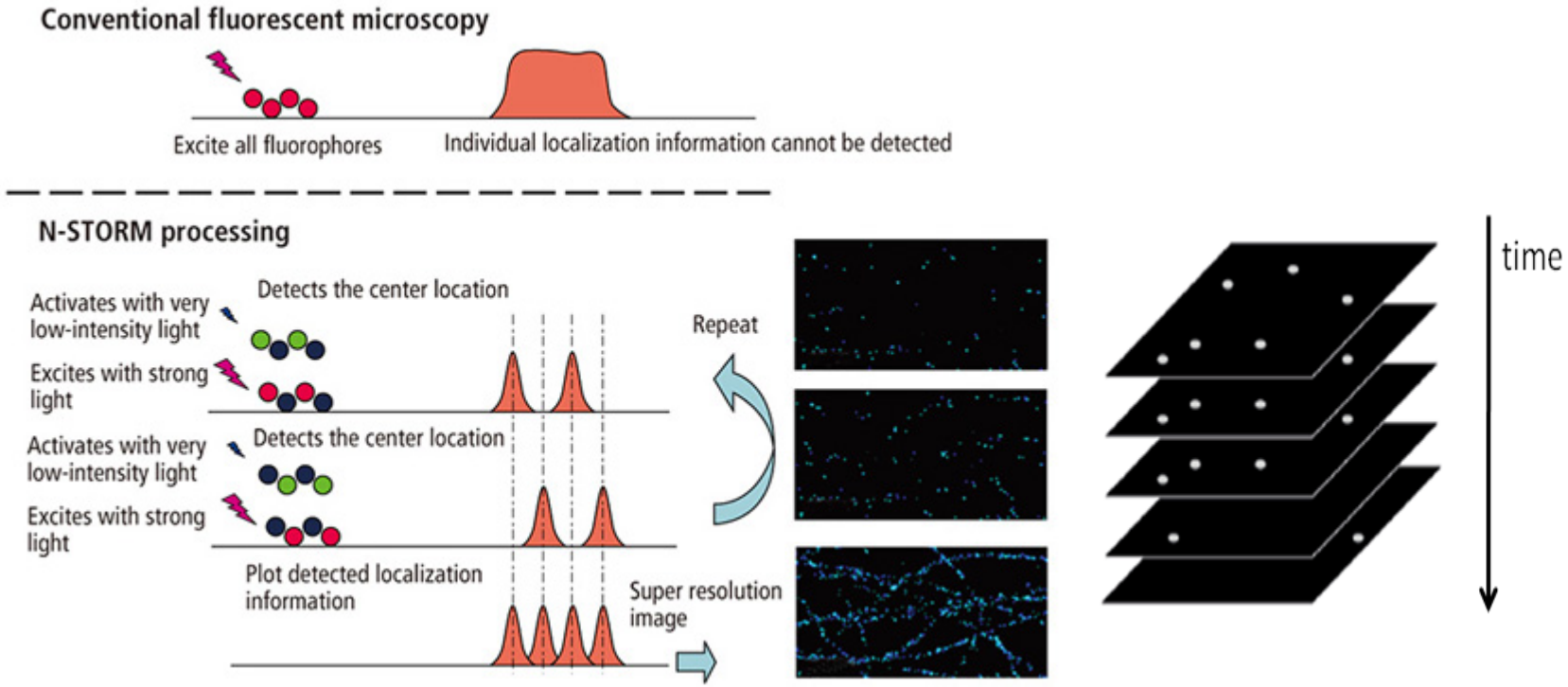
Superresolution Microscopy: Stimulated Emission Depletion (STED) Microscopy

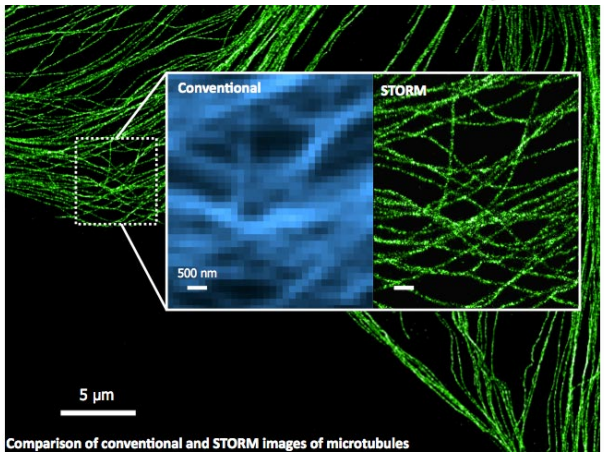


Donnert G et al. PNAS 2006;103:11440-11445

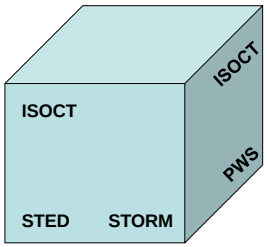


Superresolution Microscopy: Stochastic Optical Reconstruction Microscopy (STORM)





$$\sigma = \sqrt{\left(\frac{s_i^2 + \frac{a^2}{12}}{N}\right) \cdot \left(\frac{16}{9} + \frac{8\pi s_i^2 b^2}{a^2 N}\right)}$$



Nikon Superresolution Microscope

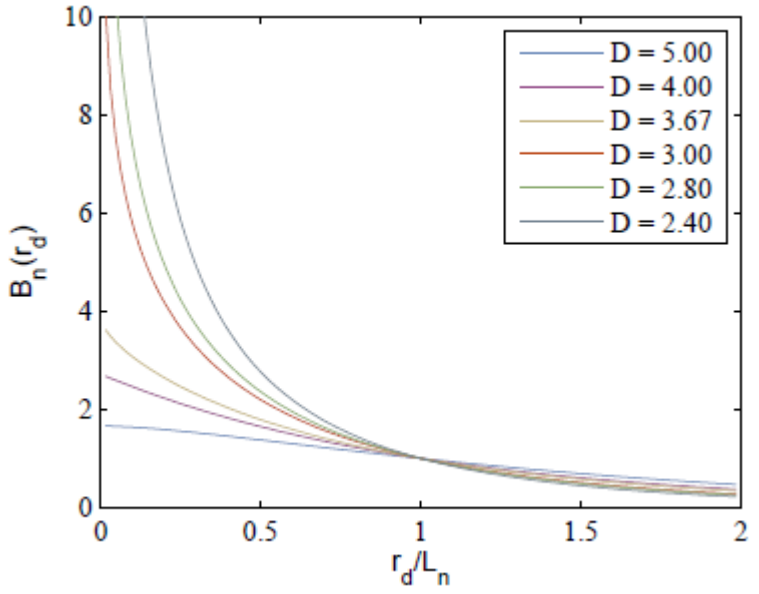
Sensing Subdiffractional Structure: What Is 'Structure'?

- Refractive index (*n*) ~ local concentration of macromolecules (ρ): $n=n_w+\alpha\rho$
- Refractive index correlation function, $B_n(r)$, formulation
- Whittle-Mattern family

$$B_n(r_d) = A_n \cdot \left(\frac{r_d}{L_n}\right)^{\frac{D-3}{2}} \cdot K_{\frac{D-3}{2}}\left(\frac{r_d}{L_n}\right)$$

Shape factor *D*:

- Gaussian: $D \to \infty$
- exponential: $D = 4$
- stretched exponential: $3 < D < 4$
- Kolmogorov / von Karman: $D = 11/3$
- Henyey-Greenstein: $D = 3$
- power law: $D < 3$



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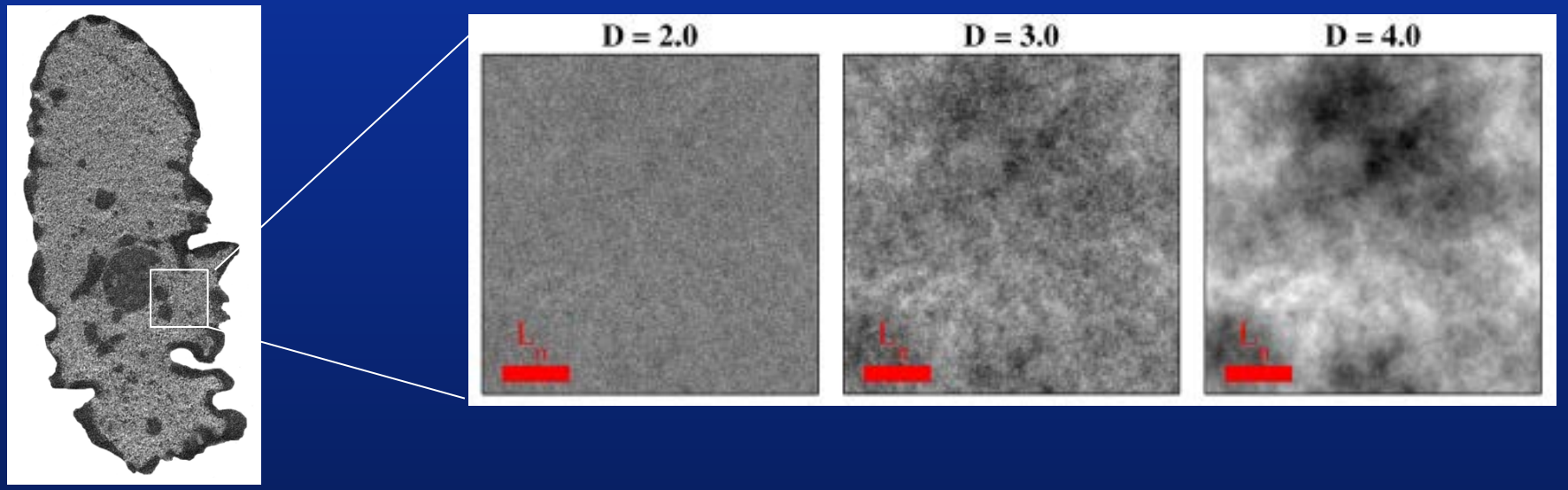
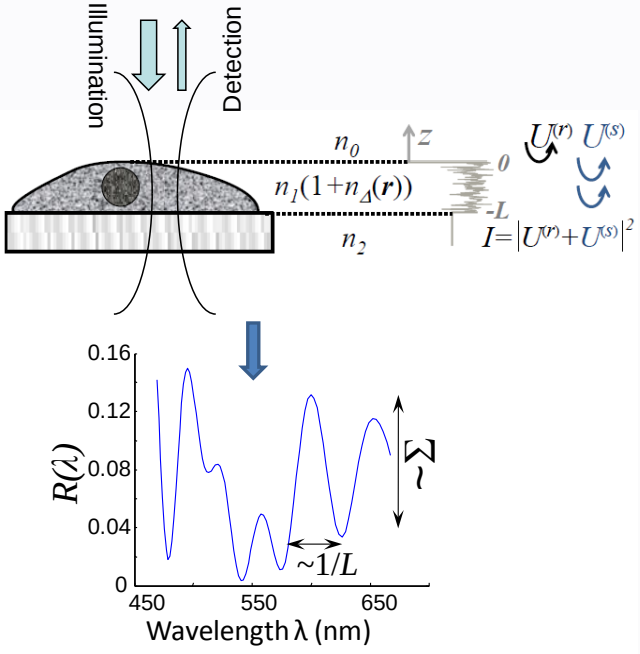


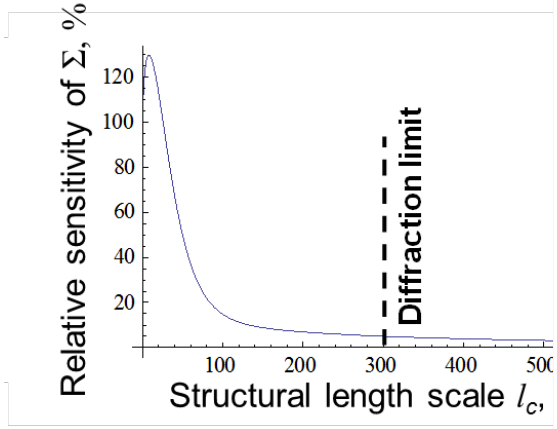
Partial Wave Spectroscopic (PWS) Microscopy: Spectroscopy + Microscopy for Nanoscale Sensing

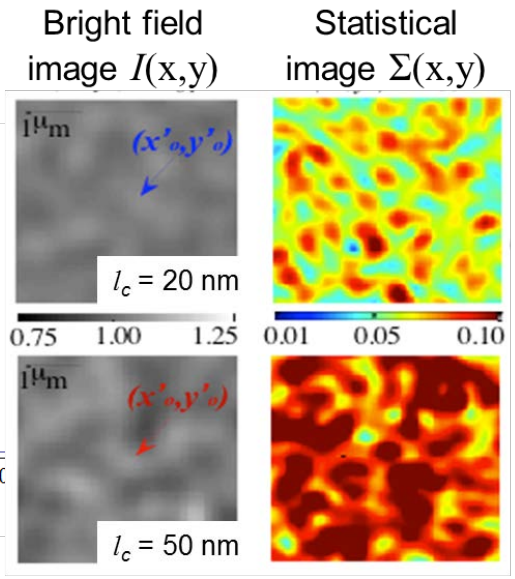
- **Physics:** Novel theory of the statistical properties of light scattering by coherence volume-restricted complex structures with nanoscale details
- **Technology:** Interference between a reference wave and light scattered by refractive index variations within a coherence volume
  - Second order spectral statistics (Σ) of coherence volume-restricted interference signal is uniquely sensitive to sub-diffractional length scales
- **Application:** Sensing intracellular nanoscale architecture

Fundamental Research: Physics



Technology: PWS





- **Not resolvable but detectable!**

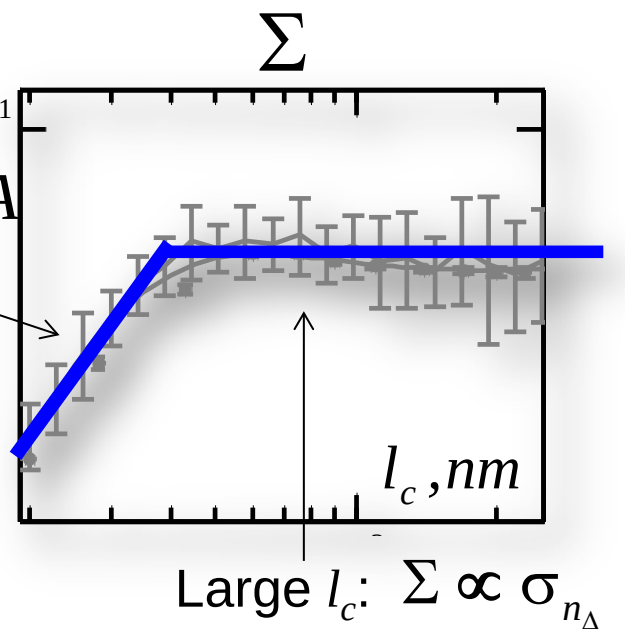
Phys. Rev. Lett. 2013

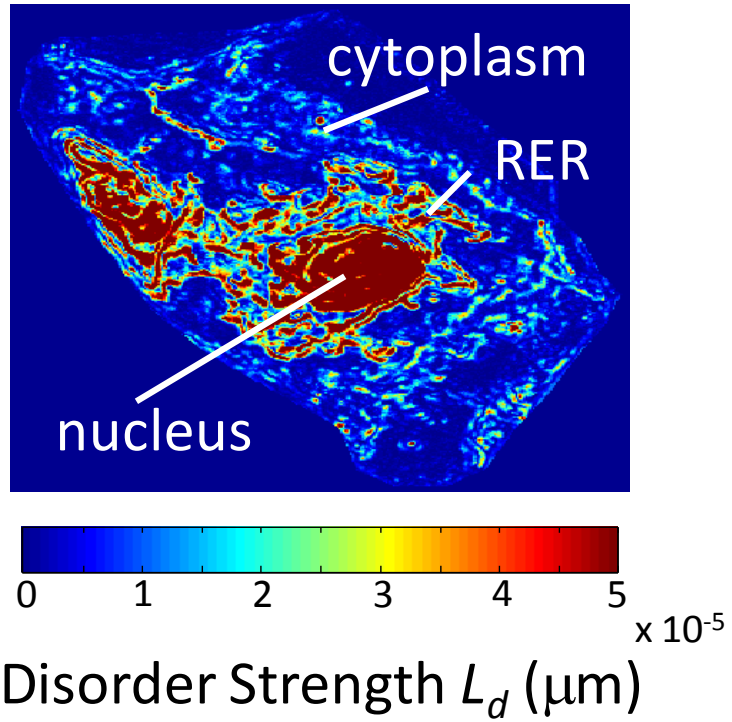
Partial Wave Spectroscopic (PWS) Microscopy: Spectroscopy + Microscopy

Small l_c:

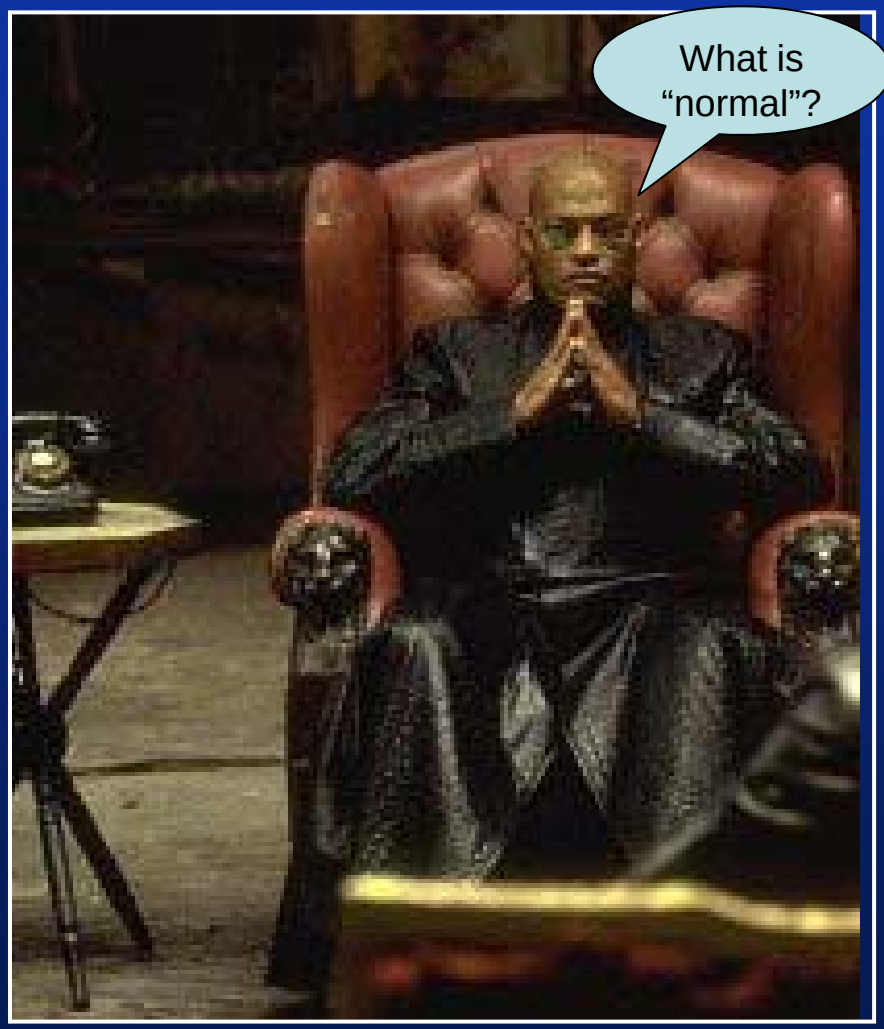
$$\Sigma \propto \underbrace{\sigma_{n_\Delta} l_c \sqrt{L}}_{L_d} NA$$

Disorder Strength of mass distribution within each pixel

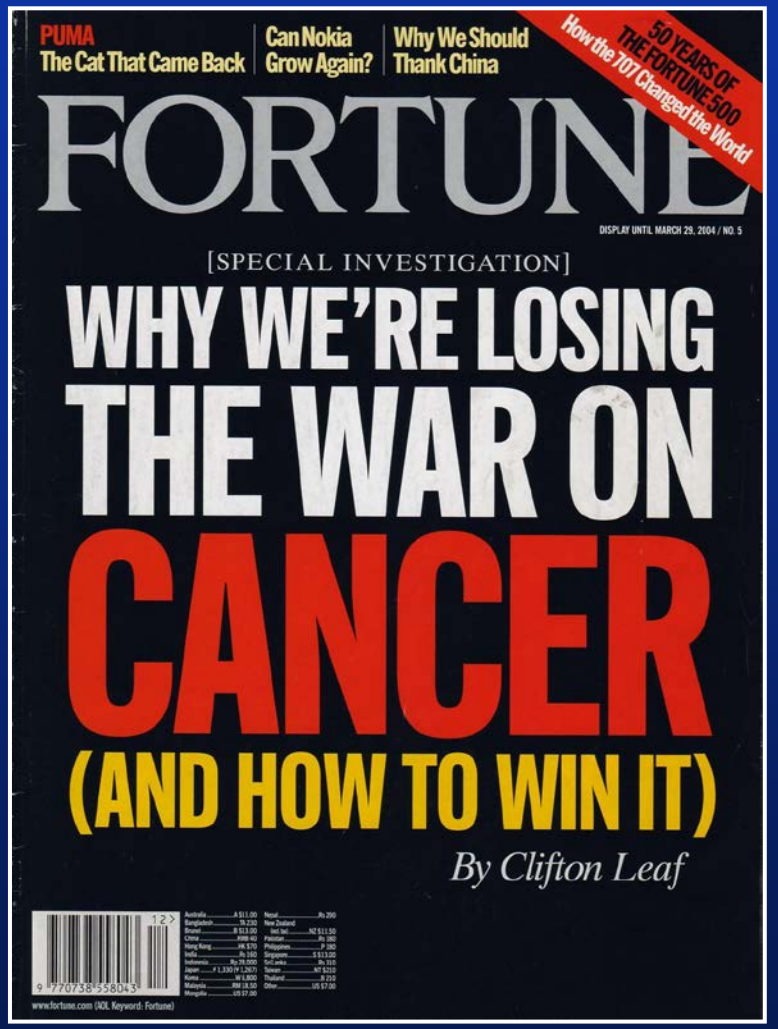




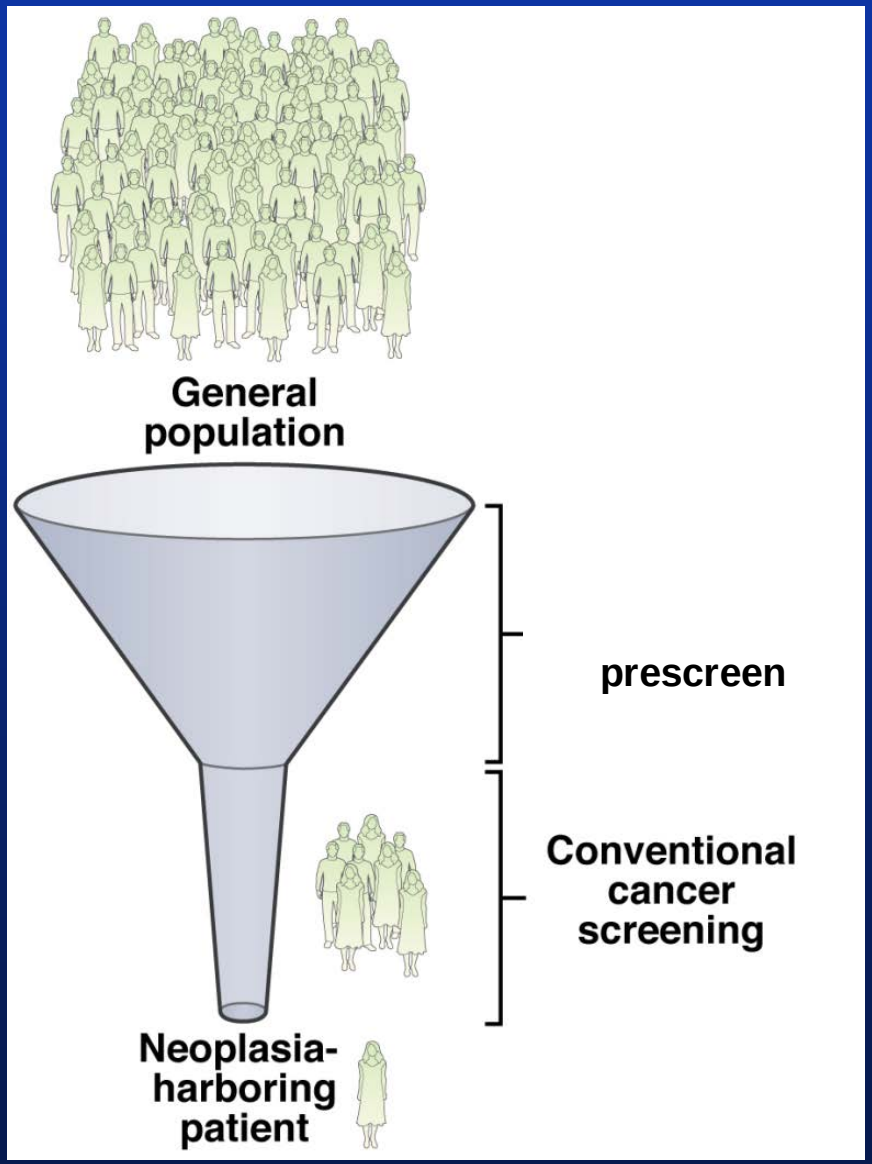
What is a (pre)-cancerous cell?



How to win the war on cancer?

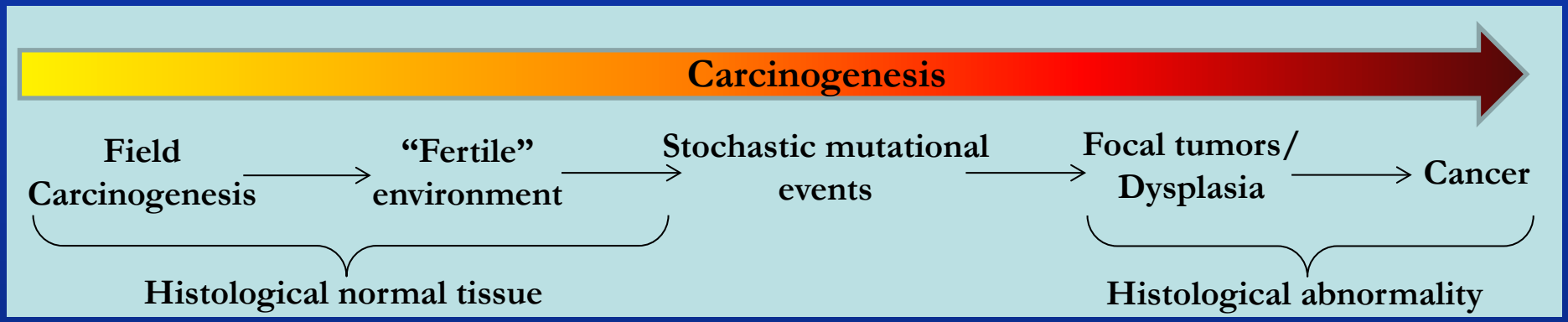


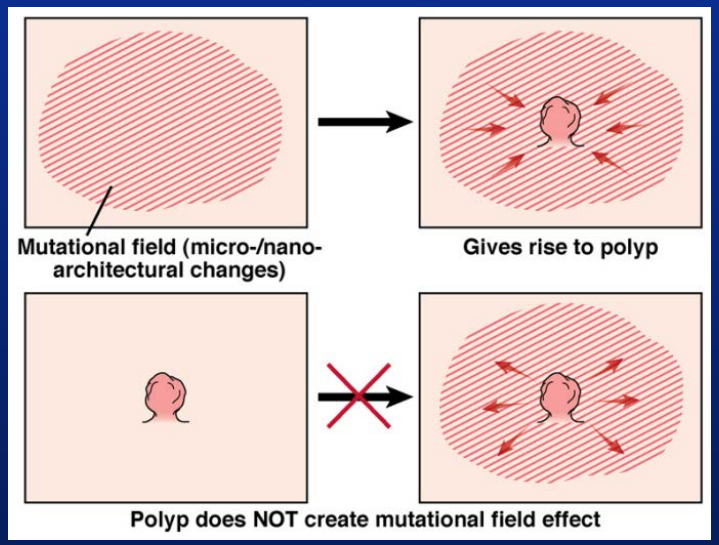
How to win the War on Cancer: Risk Stratification and Two-tier Screening

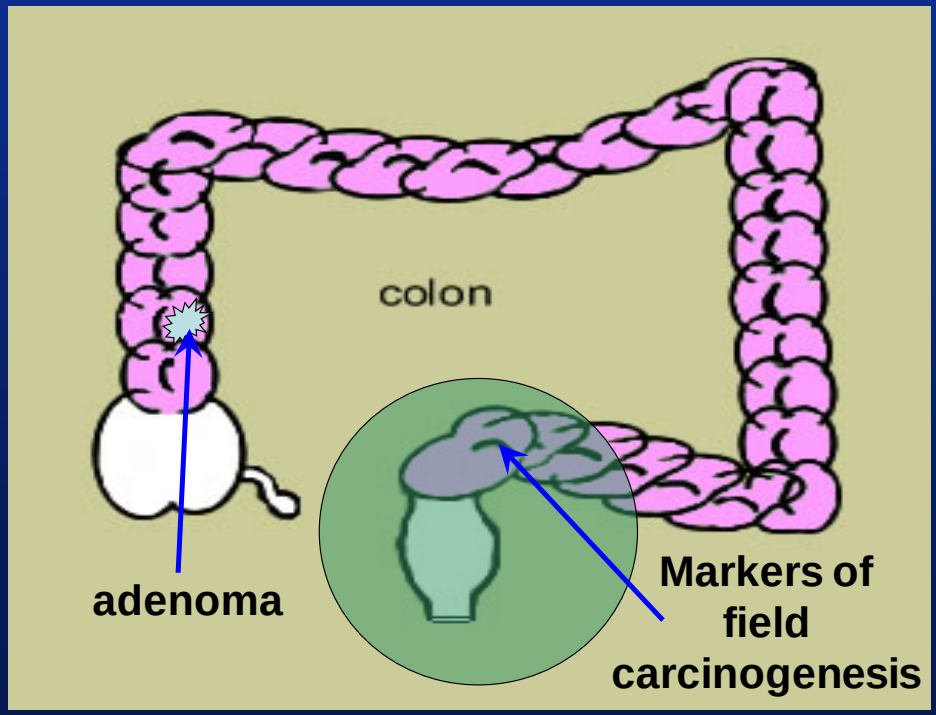


- A solution is risk stratification – a pre-screen to identify patients most likely to benefit from 2nd-tier diagnostic tests
  - Low-cost (cost effective)
  - Primary care setting
  - Non-invasive
  - No/minimal discomfort to patients
  - Sensitive not only to cancers but also to preventable lesions

Genetic / Environmental Field Carcinogenesis and Colorectal Tumorigenesis

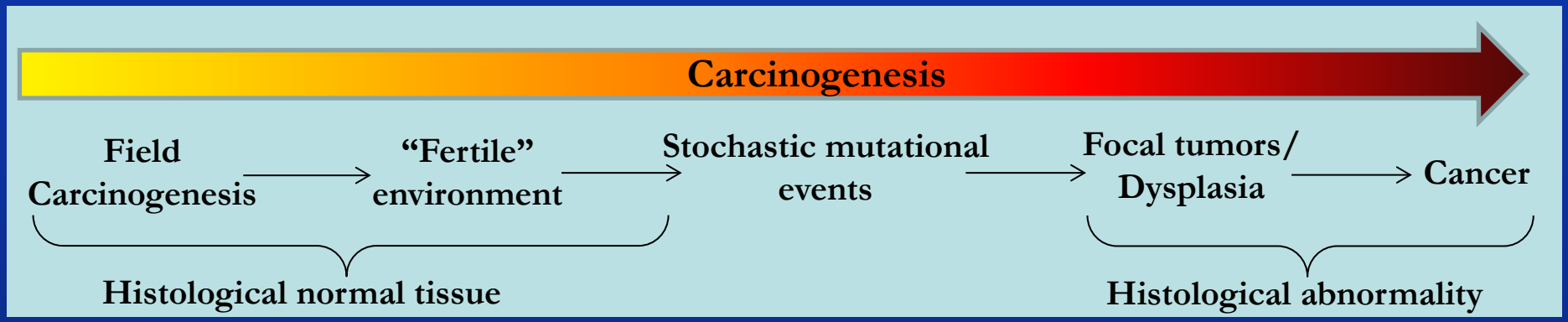


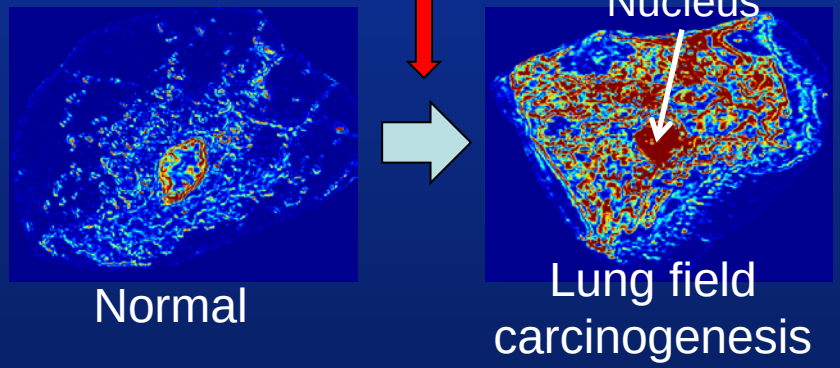


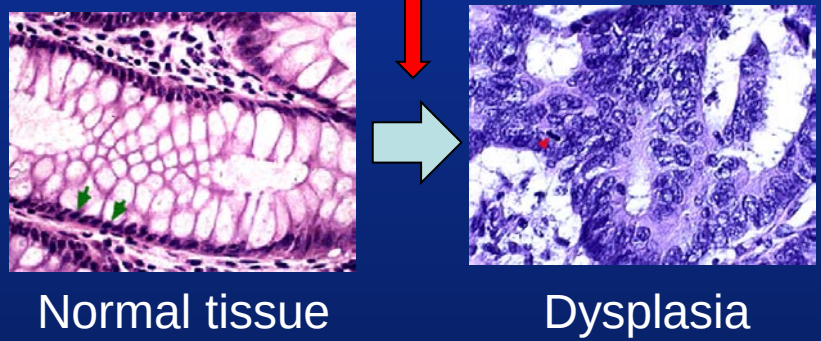


- Field effect exists in lung, head and neck, colon, breast, ovarian, esophageal, stomach, liver and other cancers.

Chromatin Alterations are One of the Best Markers of Carcinogenesis







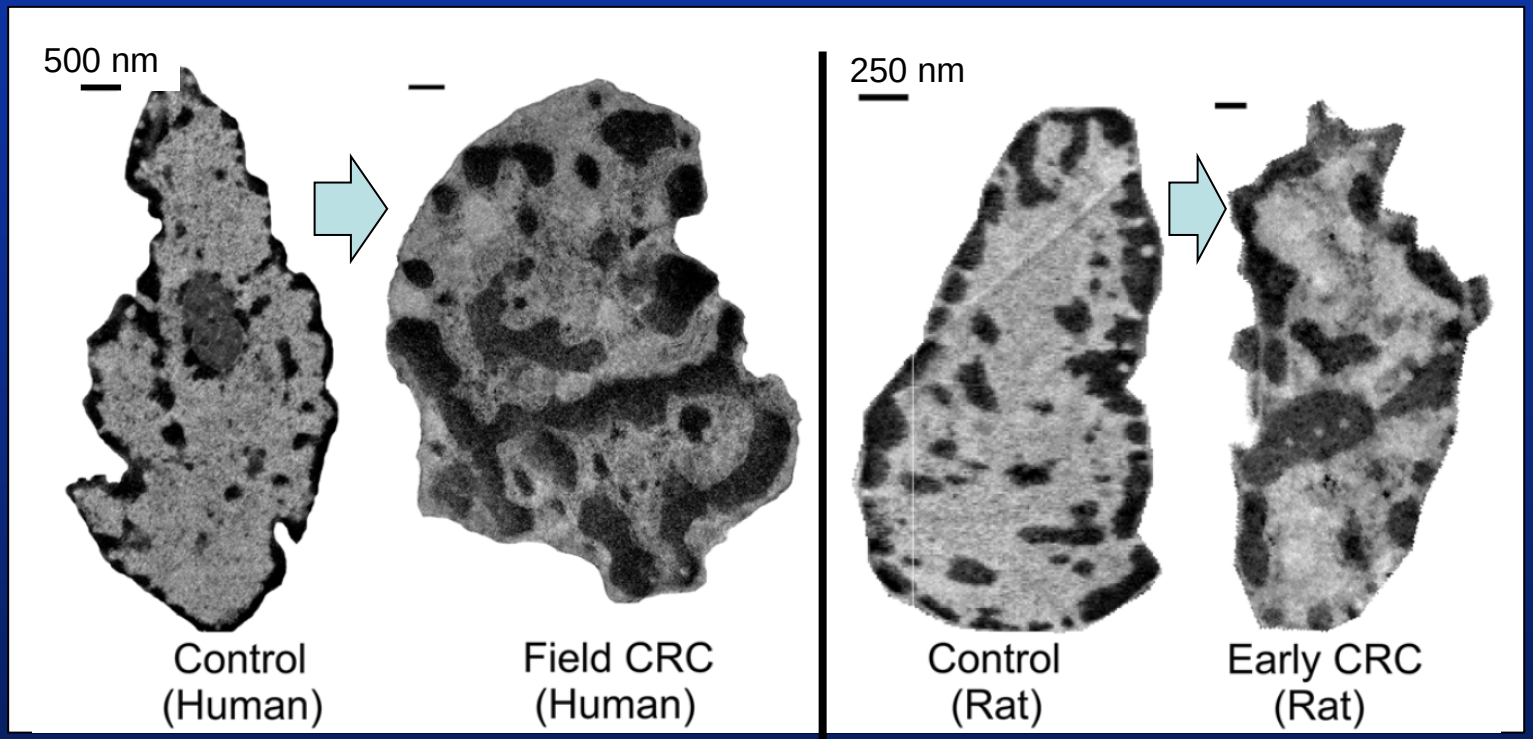
Nanoscale alterations

Microscale alterations

- **Nanocytology**: chromatin 'clumping' in histologically normal cells in field carcinogenesis precedes dysplasia
- **Histopathology**: Chromatin 'clumping' (rough/coarse chromatin) is one of the best markers of neoplasia across all solid cancers

Backman *et al., Journal of Cancer,* **4**(3), (2013)

CHROMATIN COMPACTION IN FIELD CARCINOGENESIS: TEM Evidence



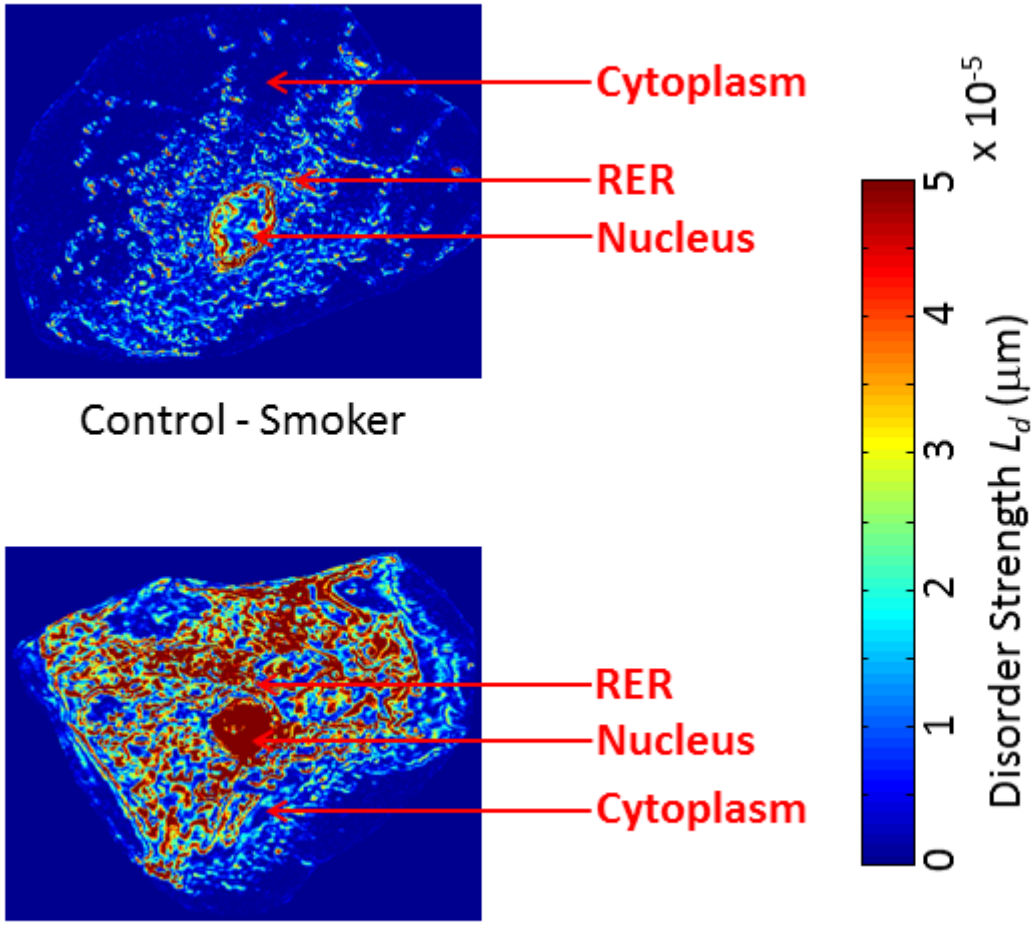
- Histologically normal rectal cell nuclei from control patients and those harboring a pre-cancerous adenoma elsewhere in the colon, representing field CRC.

- Histologically normal colonic cell nuclei from control rats and those treated with azoxymethane (10 weeks, premalignant time point), representing early CRC.

PLoS ONE (2013), *BMC* (2014)

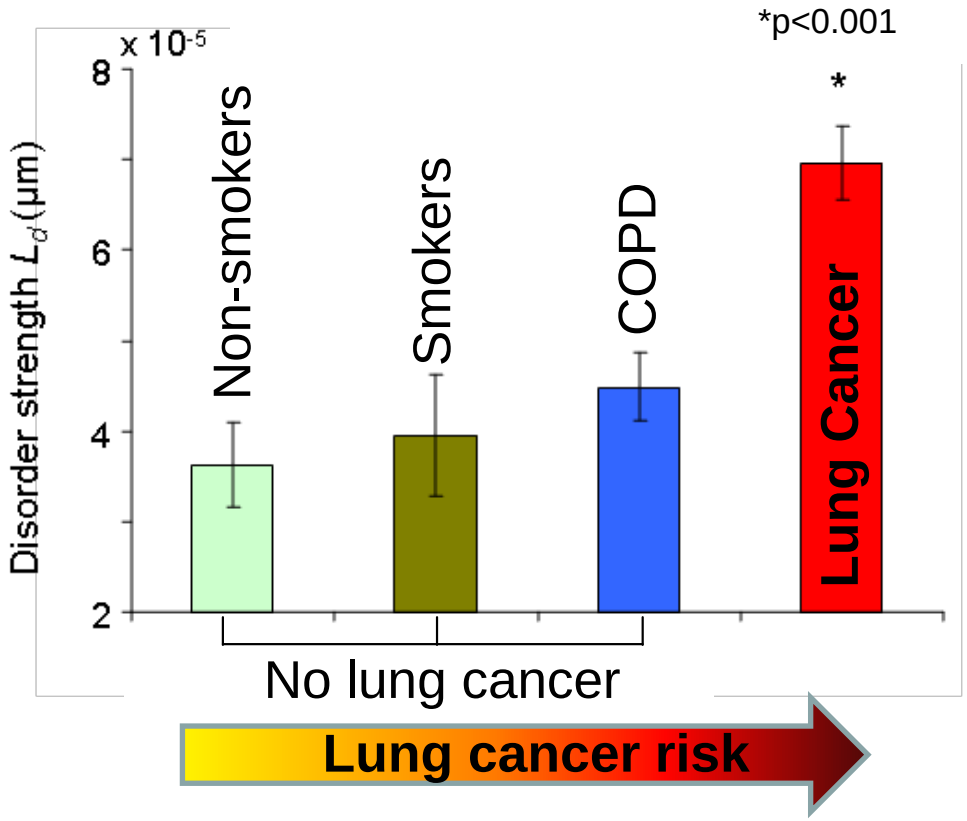
Nano-architectural Alterations in Histologically-Normal Cells in Field Carcinogenesis

Cells from buccal epithelium (cheek cells)



Cancer Research, **70**, 7748 (2010)

Disorder Strength of Histologically-Normal Buccal Cells Is Increased in Patients With Lung Cancer



STUDY DETAILS

- Blinded study design
- Cells Brushed from Buccal Mucosa
- Tumor Location: Lung
- 283 subjects
- 30 cells per patient

STUDY RESULTS

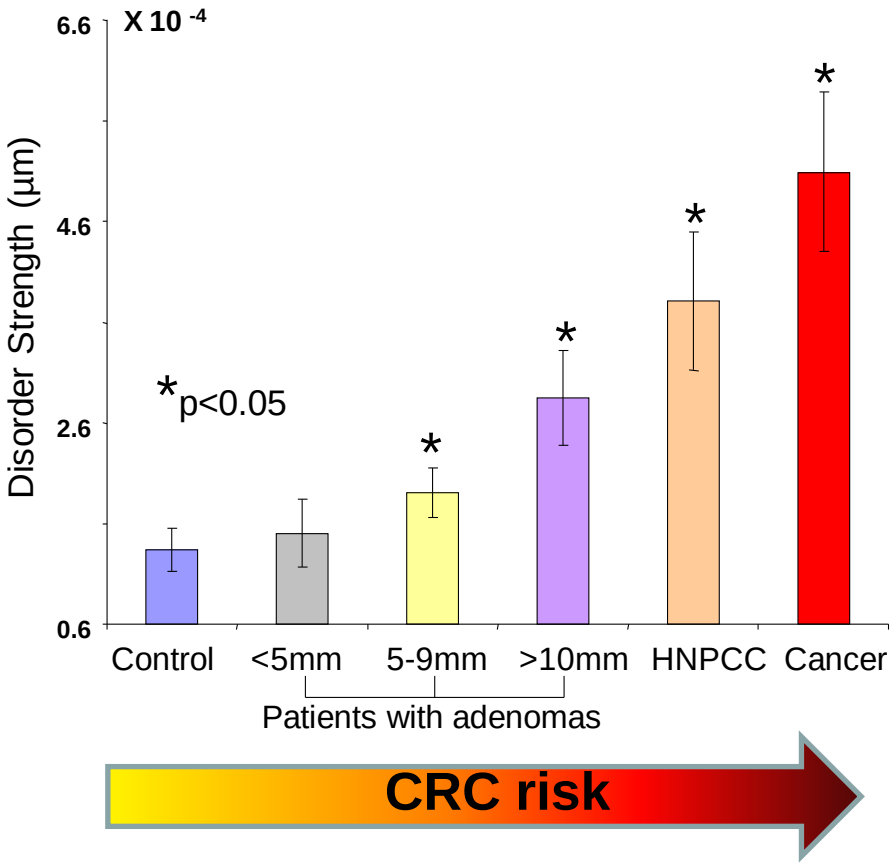
Sensitive to lung cancer irrespective of

- Stage
- Type and sub-type
- Risk and demographic factors

Nanocytology Performance: Smokers vs. lung cancer	
Sensitivity	89%
Specificity	95%

Cancer Research, ***70*** (2010)

Disorder Strength of Histologically-Normal Rectal Cells Is Increased in Patients With Adenomas Elsewhere in the Colon



STUDY DETAILS

- Blinded study design
- Cells Brushed from Rectum
- Adenoma Location: Colon
- 306 subjects
- 30 cells per patient

STUDY RESULTS

Sensitive to adenomas irrespective of

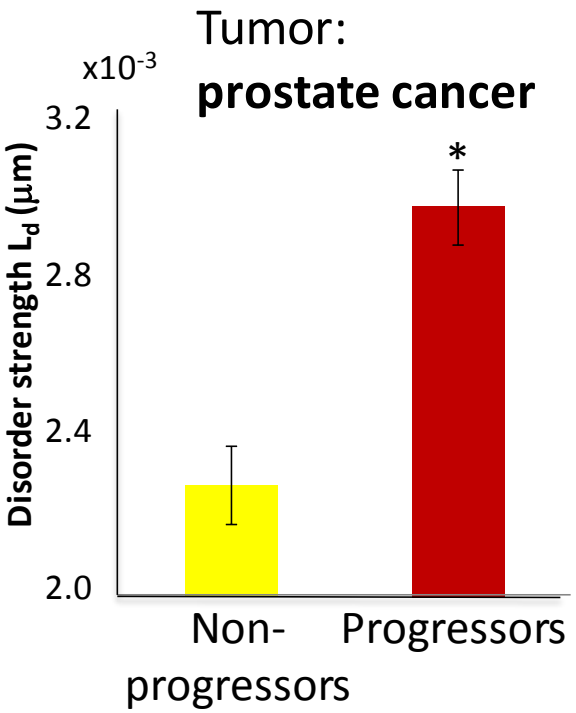
- Adenoma location
- Risk and demographic factors
- Colitis & other non-neoplastic lesions

Nanocytology for patients with advanced adenomas	
Sensitivity	85%
Specificity	85%

State-of-the-art	FOBT	FIT	Fecal DNA
Sensitivity	11%	34%	17%

Cancer Research, **72** (2012)

Disorder Strength of Prostate Cells Is Increased in Patients With Aggressive Versus Indolent Cancers



STUDY DETAILS

- Blinded study design
- Nanocytology on prostate biopsy sections
- Prostate biopsy → patients are followed to identify aggressive vs. indolent cancers
- 38 subjects

STUDY RESULTS

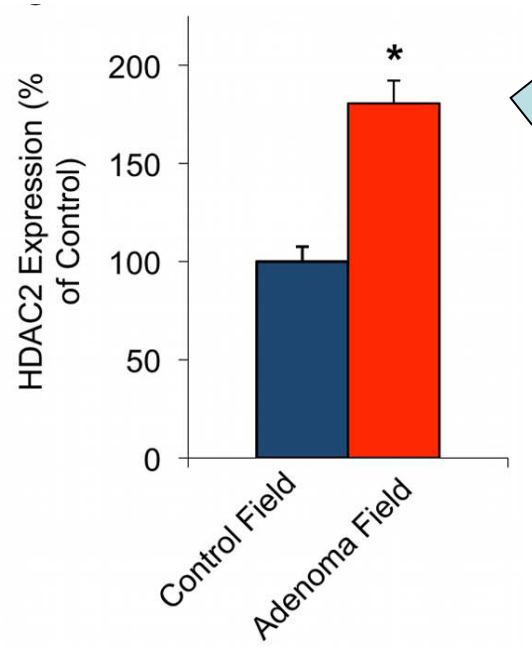
Distinguishes aggressive versus indolent prostate cancers

Nanocytology predicts aggressive versus indolent prostate cancers	
Sensitivity	80%
Specificity	88%

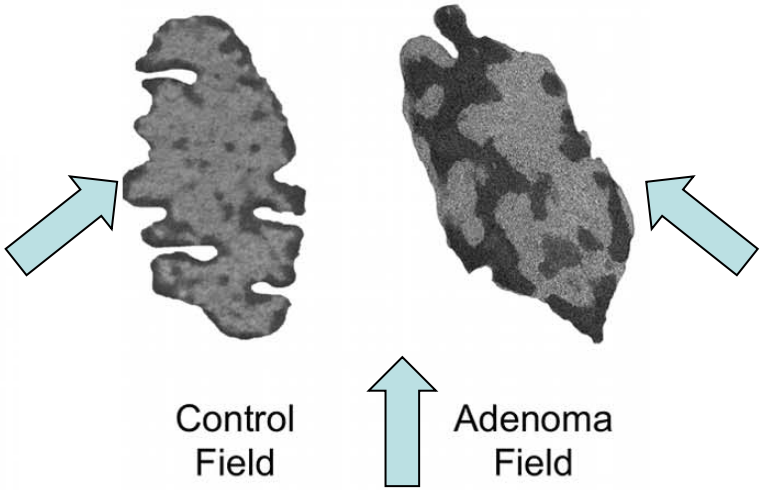
Nanoscale Alterations: A Universal Event in Carcinogenesis (7 Organ sites, 816 Subjects)

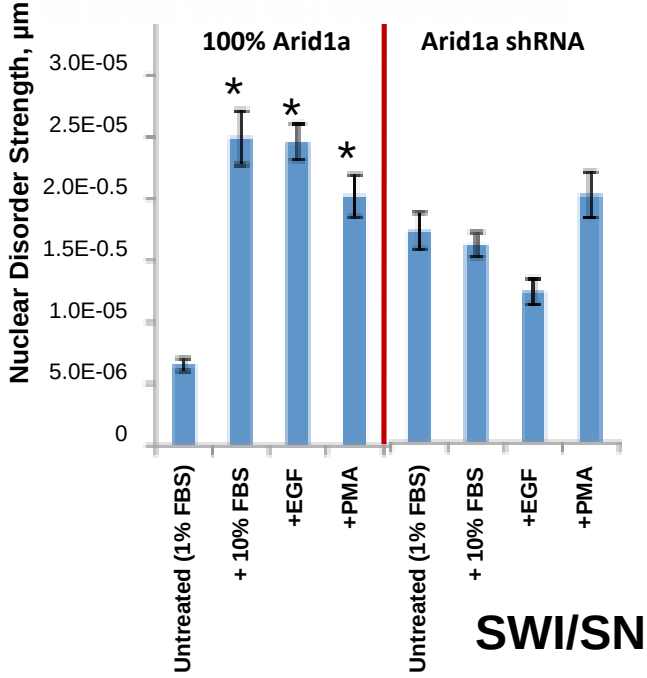
- Lung cancer: buccal cells
- Colon adenomas: rectal cells
- Prostate cancer: aggressive versus indolent cancers
- Ovarian cancer: endocervical cells
- Esophageal dysplasia: upper esophageal cells
- Pancreatic cancer: duodenal cells
- Thyroid cancer

CAUSES: Nanoarchitectural Chromatin Alterations Are a Common Outcome of Multiple Molecular Pathways

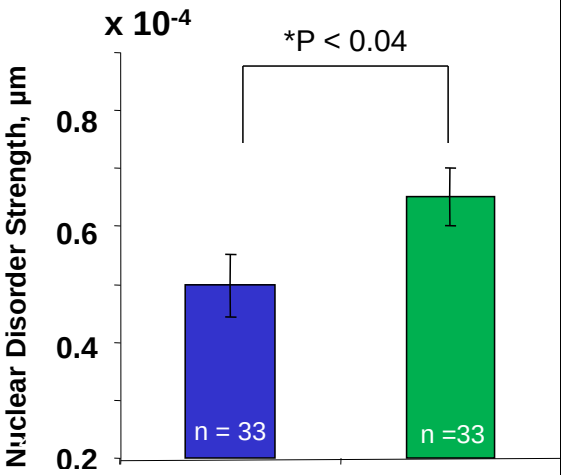


Acetylation





SWI/SNF



Methylation

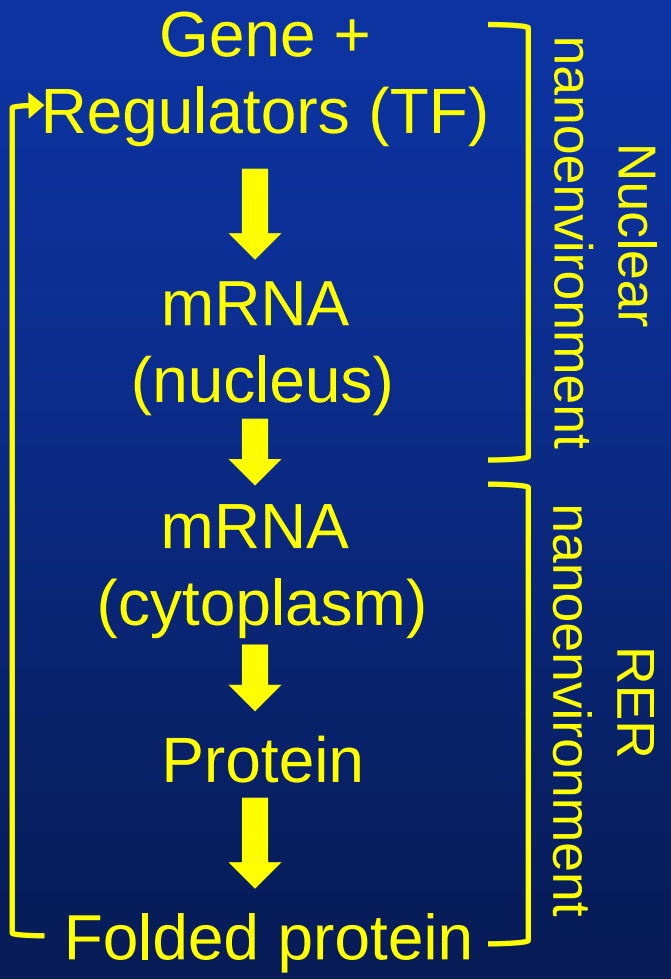
CONSEQUENCES: Nanoenvironment Affects Gene Expression

Simplified 1D gene expression process:

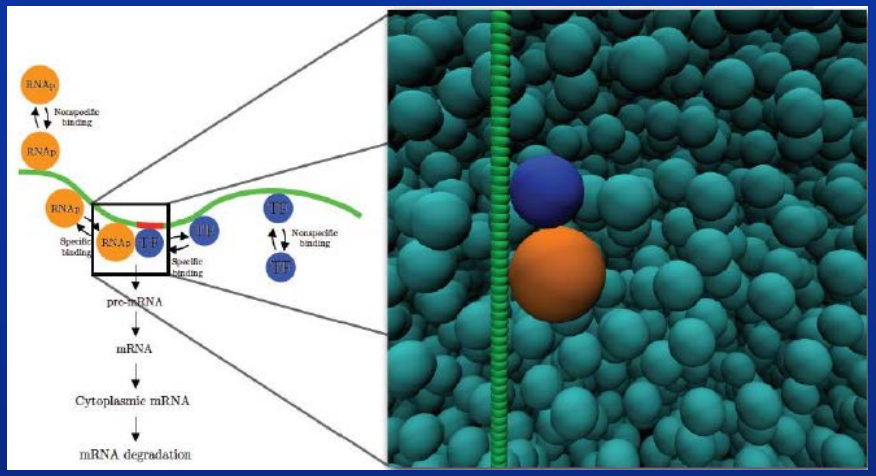
- Transcription
  - DNA-histone interaction
  - Accessibility to transcription factors
  - DNA dehybridization (DNA-helicase activity)
- Post-transcriptional modifications
  - mRNA diffusion from nucleus to RER
  - mRNA degradation by miRNA, etc.
- Translation
  - Protein synthesis
- Post-translational modifications
  - Protein folding
  - Accessibility of chaperons

3D gene expression process:

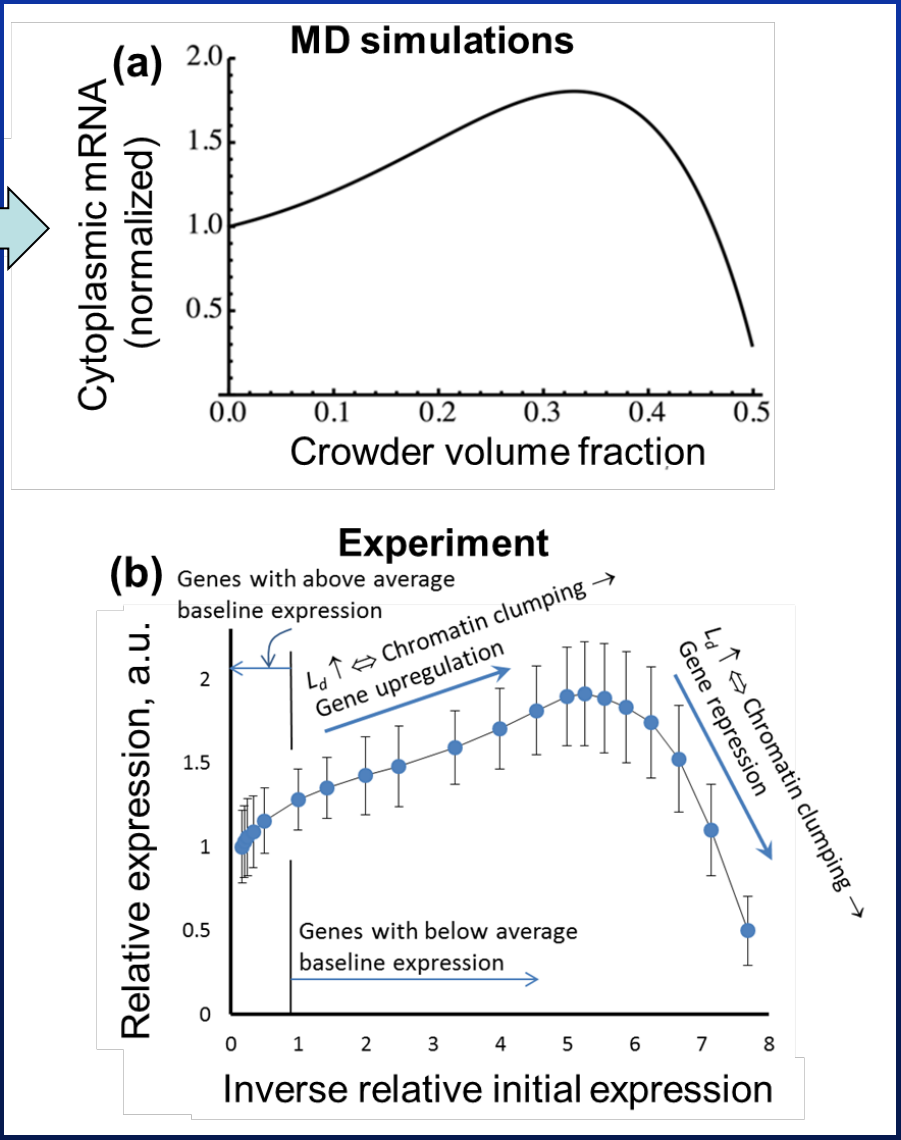
- 3D chromatin packing
- Gene co-localization and co-expression



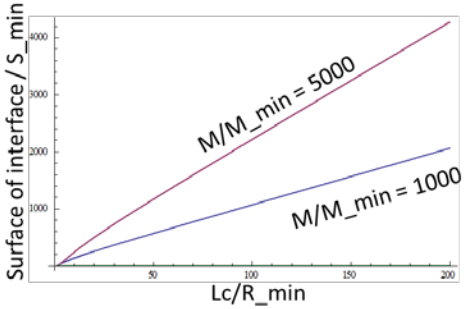
Nanoenvironment Affects Transcription Non-linearly: Systems Molecular Biophysics



- How does chromatin nanoenvironment regulate gene expression? What are the physical mechanisms?
- Molecular dynamics modeling of gene transcription in a complex nanoenvironment
- Nanoenvironment has a non-monotonic effect on gene transcription
- Other non-monotonic effects: surface area of chromatin accessible to transcription factors, work required for DNA double-helix de-hybridization



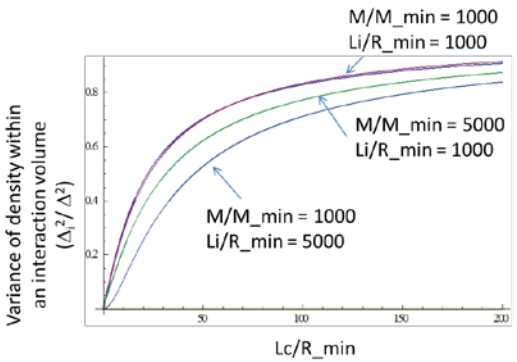
Chromatin Compaction in Early Carcinogenesis: Implications on Transcription



Chromatin heterogeneity ↑

↓

D, Ld↑



Interface surface area ↑

↓

Surface available for transcriptional interactions ↑

↓

Global transcription, global hypomethylation ↑

↓

Genomic instability

Variance of local chromatin density↑

↓

Focal highly dense chromatin ↑

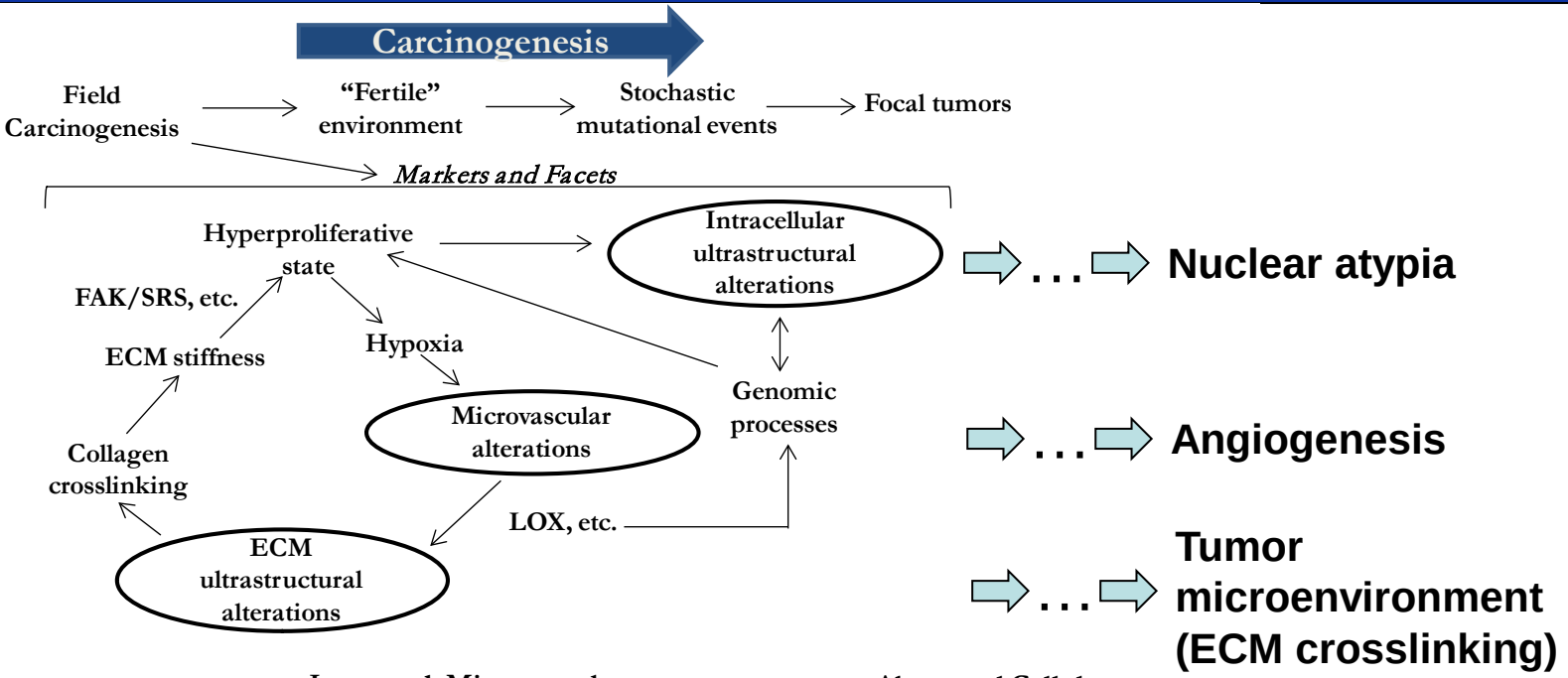
↓

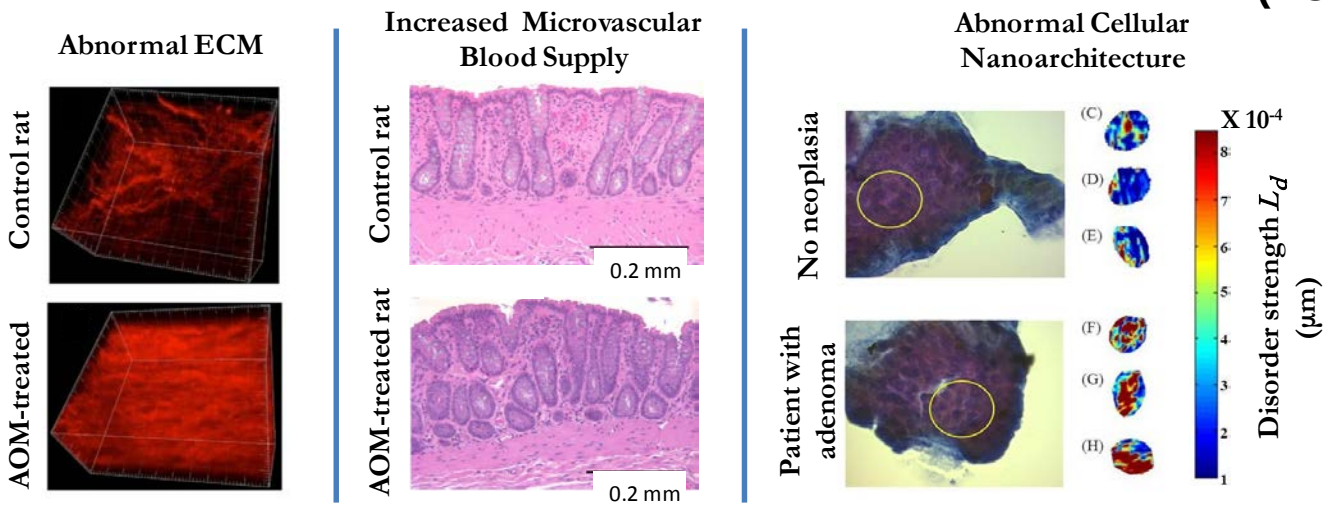
Focal gene suppression, focal hypermethylation ↑

↓

Cancer supressor genes ↓

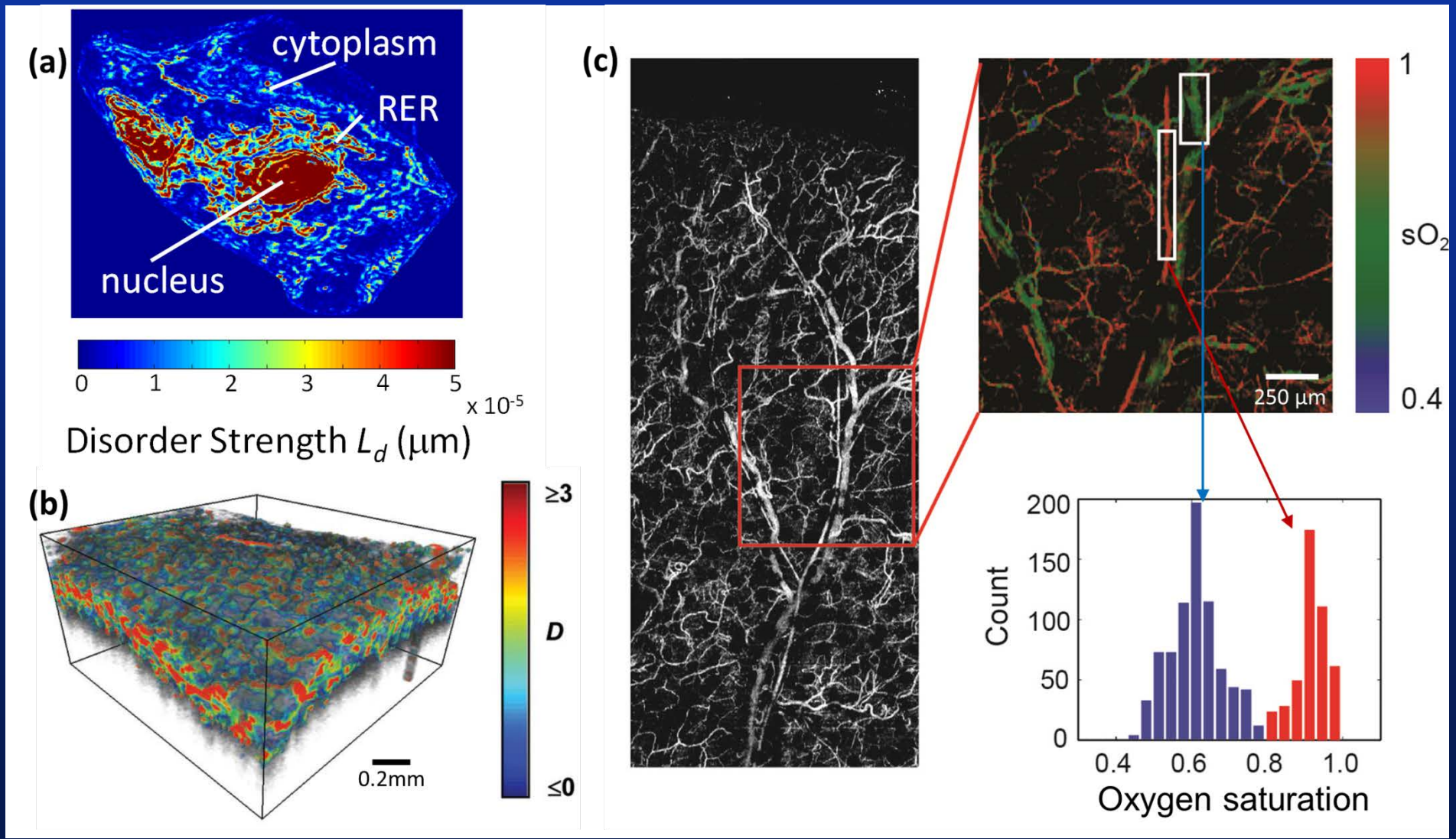
FIELD CARCINOGENESIS:
Multiple Alterations Precede Tumors



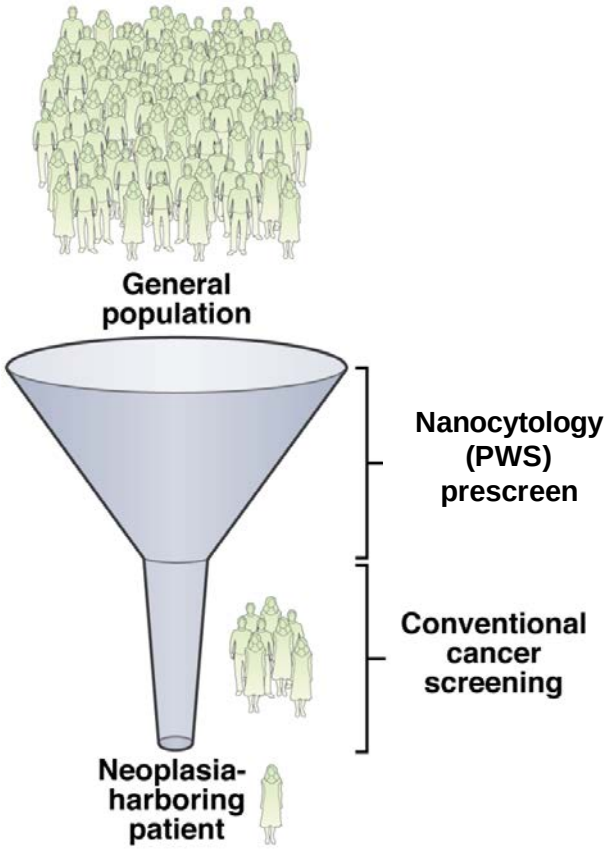


J. of Cancer ***4*** *(*2013)

Detecting ECM and Microvascular Alterations Using ISOCT



SOCIETAL IMPLICATIONS:
Population Screening



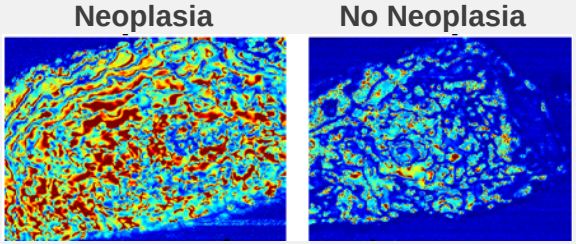
ANNUAL POPULATION SCREENING DURING ANNUAL EXAM

⇩

Cells brushed from an easily accessible surrogate site

⇩

Nanocytology prescreen: Increase in disorder of cell nanoarchitecture



⇩

Identification of patients at risk for harboring neoplasia

⇩

Most patients are screened
Patients are more compliant
Better allocation of healthcare resources

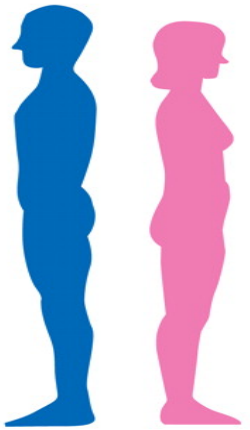
CANCER SITE	SURROGATE SITE
Colon	Rectum
Pancreas	Duodenum
Lung	Oral Cavity
Ovary	Uterus/Cervix
Esophagus	Upper Esophagus
Bladder	Urine
...	...

Approach to Population Screening

Cancer Incidence and Mortality: 2010

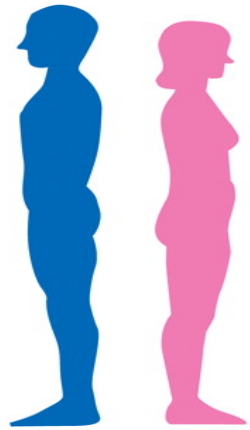
Estimated New Cases*

Males			Females		
Prostate	217,730	28%	Breast	207,090	28%
Lung & bronchus	116,750	15%	Lung & bronchus	105,770	14%
Colon & rectum	72,090	9%	Colon & rectum	70,480	10%
Urinary bladder	52,760	7%	Uterine corpus	43,470	6%
Melanoma of the skin	38,870	5%	Thyroid	33,930	5%
Non-Hodgkin lymphoma	35,380	4%	Non-Hodgkin lymphoma	30,160	4%
Kidney & renal pelvis	35,370	4%	Melanoma of the skin	29,260	4%
Oral cavity & pharynx	25,420	3%	Kidney & renal pelvis	22,870	3%
Leukemia	24,690	3%	Ovary	21,880	3%
Pancreas	21,370	3%	Pancreas	21,770	3%
All Sites	**789,620**	**100%**	**All Sites**	**739,940**	**100%**



Estimated Deaths

Males			Females		
Lung & bronchus	86,220	29%	Lung & bronchus	71,080	26%
Prostate	32,050	11%	Breast	39,840	15%
Colon & rectum	26,580	9%	Colon & rectum	24,790	9%
Pancreas	18,770	6%	Pancreas	18,030	7%
Liver & intrahepatic bile duct	12,720	4%	Ovary	13,850	5%
Leukemia	12,660	4%	Non-Hodgkin lymphoma	9,500	4%
Esophagus	11,650	4%	Leukemia	9,180	3%
Non-Hodgkin lymphoma	10,710	4%	Uterine Corpus	7,950	3%
Urinary bladder	10,410	3%	Liver & intrahepatic bile duct	6,190	2%
Kidney & renal pelvis	8,210	3%	Brain & other nervous system	5,720	2%
All Sites	**299,200**	**100%**	**All Sites**	**270,290**	**100%**



Clinical data

Potential/planned

Jemal, A. et al. CA Cancer J Clin 2010

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