Nanomedicine: State of the Art and Emerging Opportunities

Jim Heath, Caltech

Quantum Dots as Biological Labels

(high profile; relatively low impact bio-application)

Quantum size effects in colloidal particles

Bell labs: **early 1980's**

Surface Science of Semiconductor QDs

Bell Labs, MIT, UC Berkeley, UCLA, Germany: **early 1990's**

Synthetic advances to produce monodisperse QDs

concepts (Bell labs, **1950's**); practice (MIT, **1994**)

III-V QD materials

Colorado, Berkeley, UCLA **mid 1990's**

Core-shell QDs (highly efficient, non-bleaching fluorophores)

Chicago, Berkeley, MIT **1996-2000**

QD Biolabels (Indiana University & Berkeley, **1996, 1997**)

QD Corporation (1998) (out of Berkeley, MIT, IU)

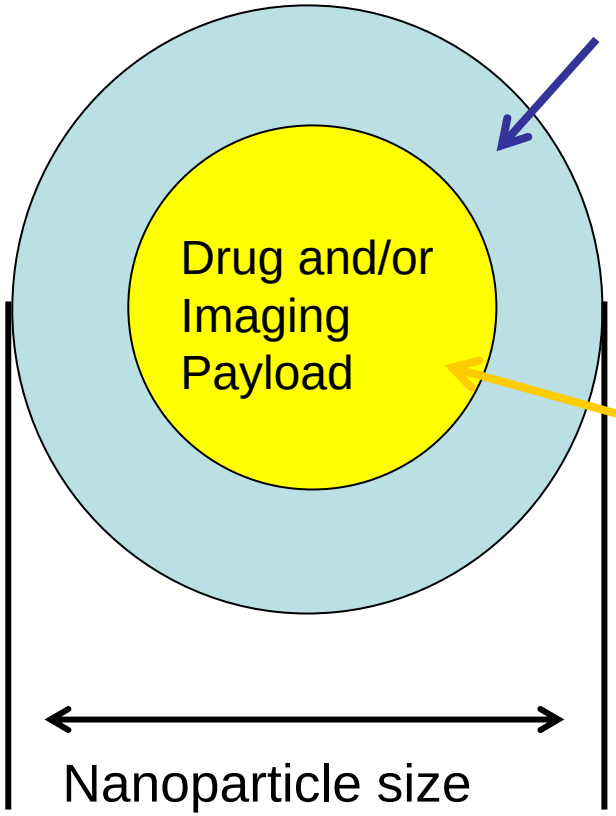
Commercially available QDs (~2004)

Clincial applications (in vitro diagnostics of breast cancer)

Ga Tech/Emory; UCSF, ? 2008

Nanoparticle Therapeutics

High profile nanotechnology, high impact application



Nanoparticle matrix & surface charge

- increase solubility of drug
- minimize capture by liver cells
- minimize protein adhesion (biofouling)
- disease-specific targeting
- optimized cell wall passage
- minimize immuno response

Drug or Imaging payload

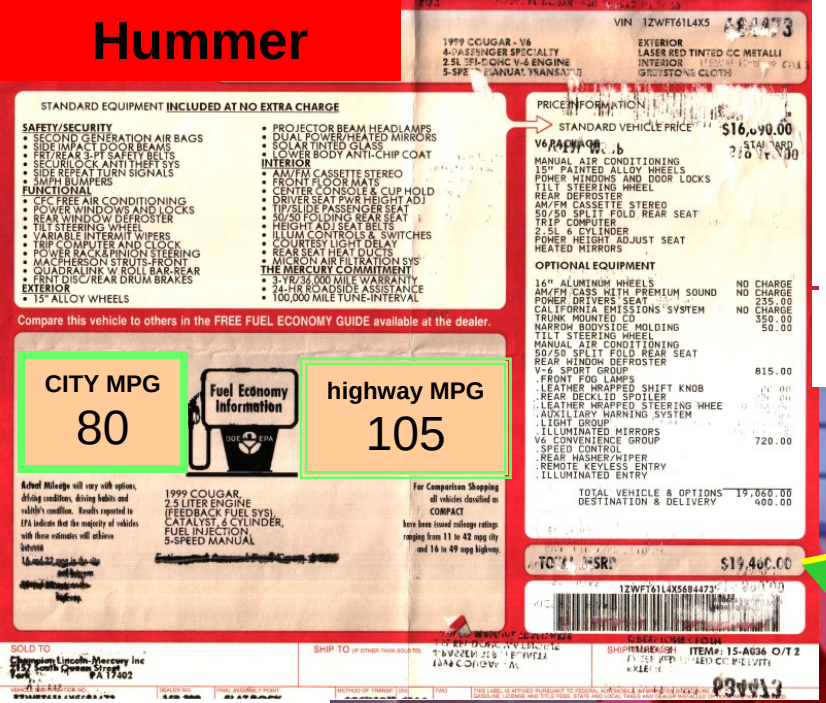
- Approved drug (chemotherapy agents)
- siRNA, gene therapy agent, etc.
- PET probe (^{64}Cu or ^{18}F-labeled probe)
- MRI contrast agent, fluorophore

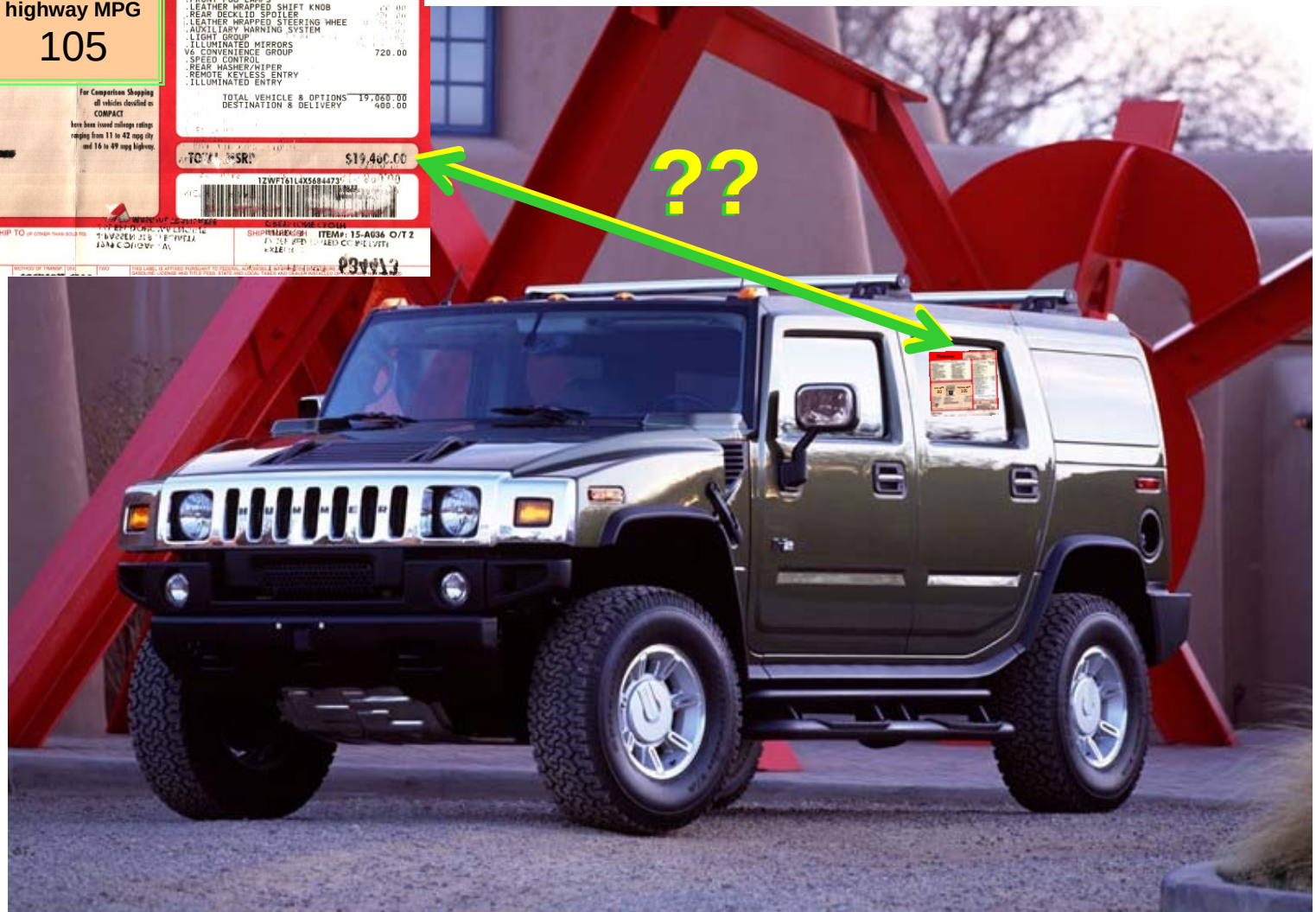
- increase circulation times 1 to 100 fold
- optimize traversal through tumor
- large drug payload (10-2000 molecules/particle)

Hummer

The best of nanomedicine is science & technology that is solving problems like this one

BIG, FAST & EFFICIENT





NANOSYSTEMS BIOLOGY
N S B
C C
CANCER CENTER

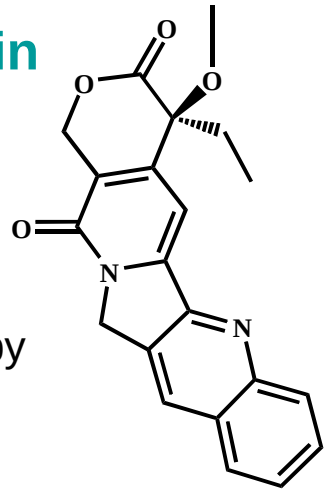
CALIFORNIA INSTITUTE OF TECHNOLOGY
1891

A big/fast/efficient problem

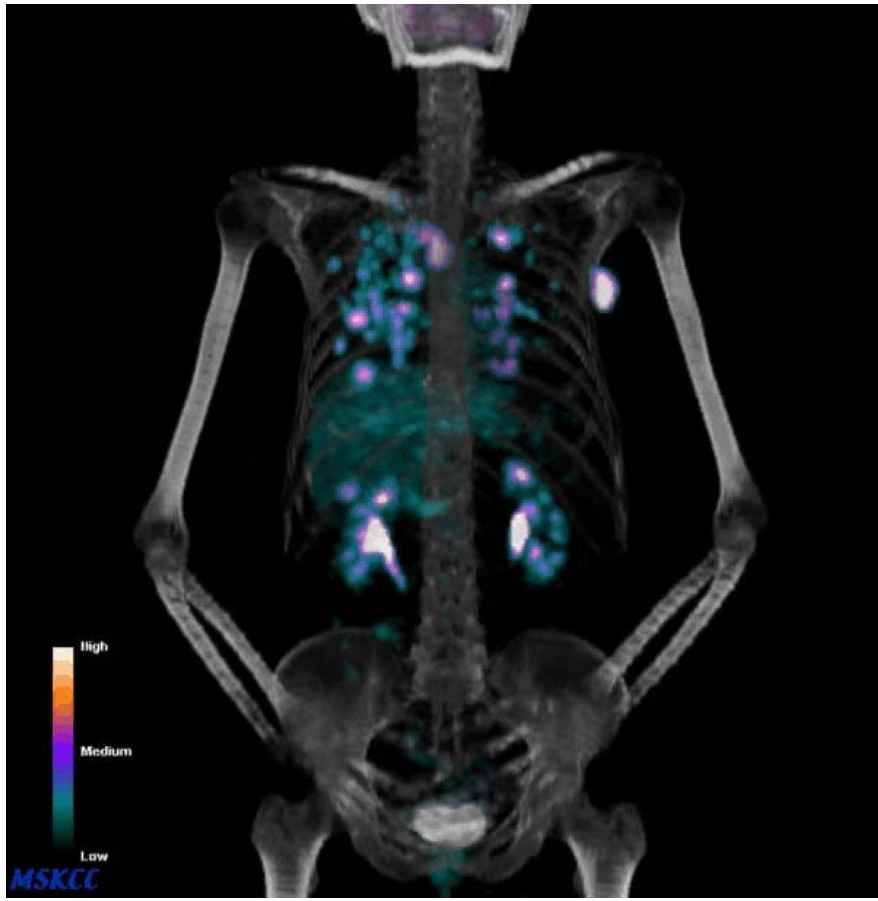
How we want to treat this cancer patient

- **Inject or swallow a drug**
- **Deliver only to the cancer**
- **Kill the cancer cells**
- **Cause no toxicity**

Camptothecin



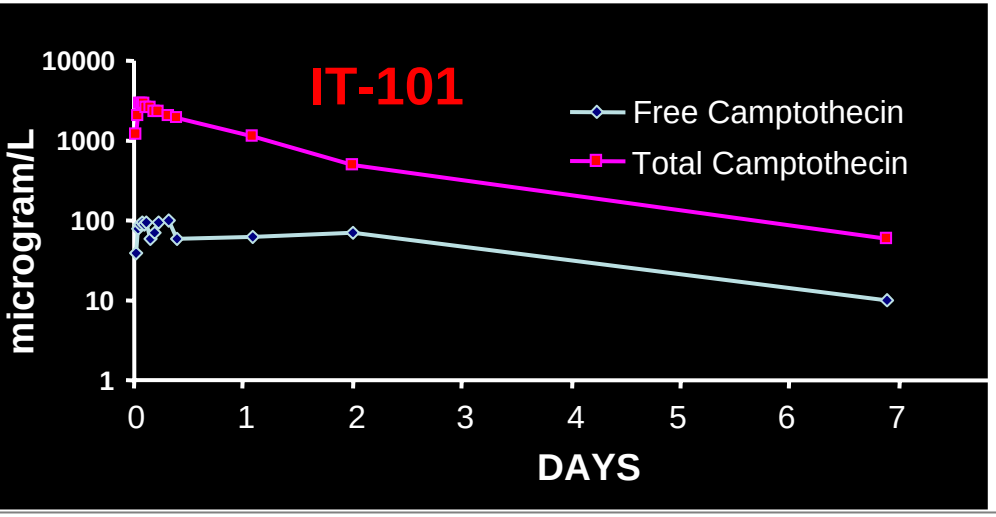
A chemotherapy agent



Normal solution

- **Inject drug**
- **Kill all fast growing cells**
- **Deal with high toxicity of drug**

Nanosolution: Design a nanoparticle to deliver the drug

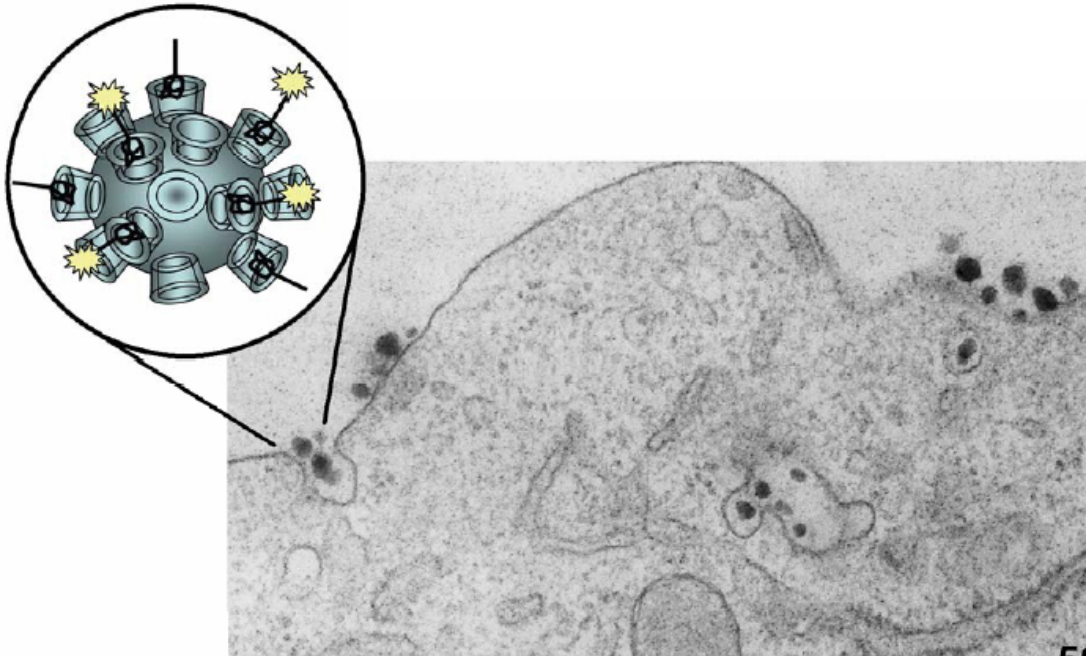


PK in humans : T. Synold, Y. Yen at City of Hope, CA

- Most drugs clear within ~1 hour
- NP's circulate for days

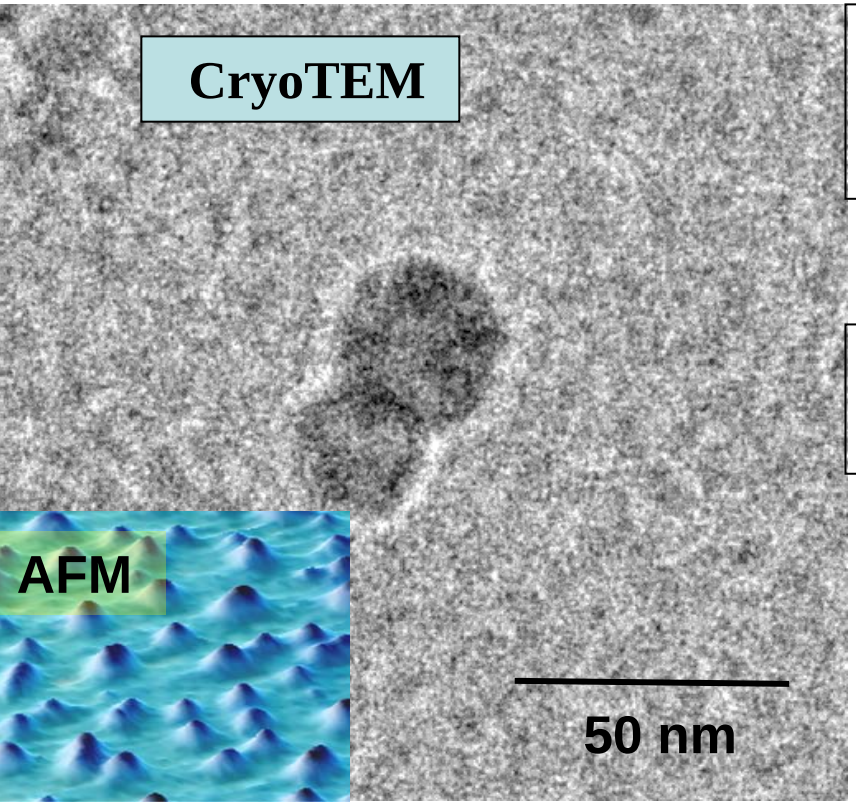
Large dose reduction lower toxicity
higher efficacy per mg drug

- NP's have a high payload (10^3 drug molecules per particle)



Prof. Mark Davis
Chemical Engineering
Caltech
& Insert Therapeutics, Inc.

IT-101: Size and Surface Charge by Design



Particle Diameter

Dynamic Light Scattering: 30-40 nm
CryoTEM: 25-35 nm

Particles < 20 nm: filtered out quickly by kidneys
Particles > 80 nm: trapped in liver

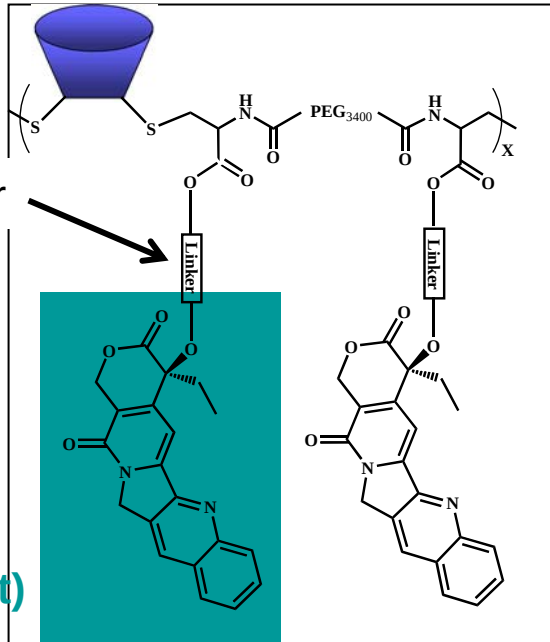
Surface Charge

Zeta Potential = - 6.3 +/- 2.4 (in PBS)

Positively Charged: Don't circulate well
Too negatively charged: immune system destroys

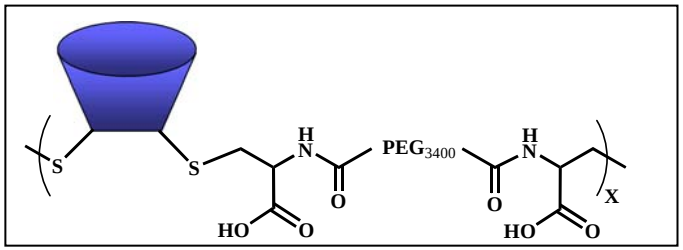
Acid-cleavable linker

(tumor microenvironment catalyzes release of drug)



Polycyclodextrin carrier

- **No immune response**
- **Not metabolized**
- **Low toxicity**



Camptothecin (anti-tumor agent)

Nano-scaled systems for systemic therapy

Platform	Latest stage of development	Examples
liposomes	approved	DaunoXome, Doxil®
albumin-based particles	approved	Abraxane
nanocrystals	approved	Rapamune (oral), Emend (oral)
polymeric micelles	clinical	Genexol-PM, SP1049C, NK911, NK012
polymer-based particles	clinical	XYOTAX, IT-101, CT-2106, AP5346
dendrimers	preclinical	polyamidoamine (PAMAM)
inorganic or other solid particles	preclinical.preclinical, clinical	carbon nanotubes, silica particles, gold particles

From J. Heath, M. Davis. *Ann. Rev. Medicine* (in press, 2007)

Table 2 Comparison of pharmacokinetics (human) of small-molecule drugs to nanoparticle therapeutics

Name	Formulation[a]	Diameter (nm)	Clearance rate (ml/min•kg)	Reference
DOX	0.9% NaCl	---	14.4	80
SP1049C	pluronic micelle/DOX	22--27	12.6	80
NK911	PEG-Asp micelle/DOX	40	6.7	80
Doxil®	PEG-liposome/DOX	80--90	0.02	80
Taxol®	Cremophor® EL	---	3.9 (9.2)	79, 80
Genexol	PEG-PLA micelle/paclitaxel	20--50	4.8	80
Abraxane	albumin/paclitaxel	120[b]	6.5	79
XYOTAX	PG/paclitaxel	?	0.07--0.12	82
LE-SN38	liposome/SN-38	?	3.5--13.6	83
CT-2106	PG/CPT	?	0.44	84
IT-101	CD polymer/CPT	30--40	0.03	85

[a]Abbreviations: DOX, doxorubucin; PEG-PLA, block copolymer of polyethylene glycol-poly(L-lactic acid); PG, polyglutamic acid; CPT, camptothecin; CD polymer, cyclodextrin-containing polymer
[b]Dissolves upon exposure to blood

From J. Heath, M. Davis. *Ann. Rev. Medicine* (in press, 2007)

Clinical Pathways of Nanoparticles

IT-101 Nanoparticles manufactured at ~ 5 kg scale

- IT-101: Phase I/Ib for treating solid tumors refractory to standard therapy or for which no standard therapy exists : City of Hope Cancer Center (1st patient dosed 7/06)
- IT-101: Phase II clinical trials in early 2008
- CALAA-01: Phase I for the treatment of solid tumors refractory to standard therapy or for which no standard therapy exists planned to begin late 2007 at City of Hope and UCLA. First targeted nanoparticle with siRNA and first nanoparticle with siRNA for cancer.

Meeting phase I FDA metrics using standard clinical testing labs is challenging because industry is not prepared to measure the appropriate metrics

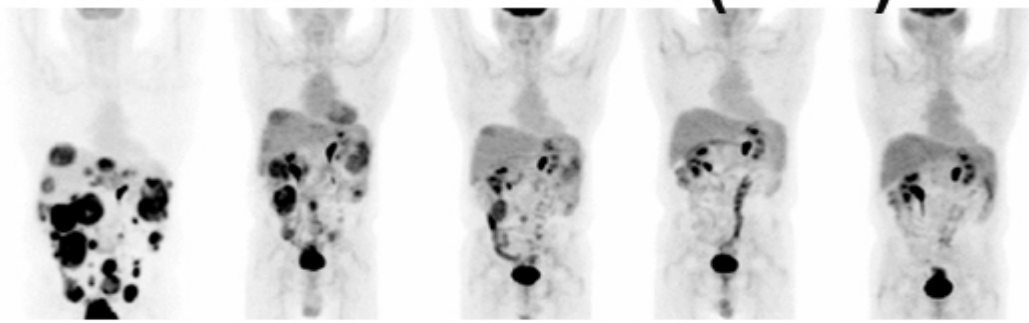
Case study: **Patient with late stage, non-treatable pancreatic cancer – many metastasis to lungs, etc., 2-3 months survival prognosis**
Treated 1 year ago; healthy and in remission today

Nanomedicine: *in vitro* diagnostics

Images: Dana Farber CC

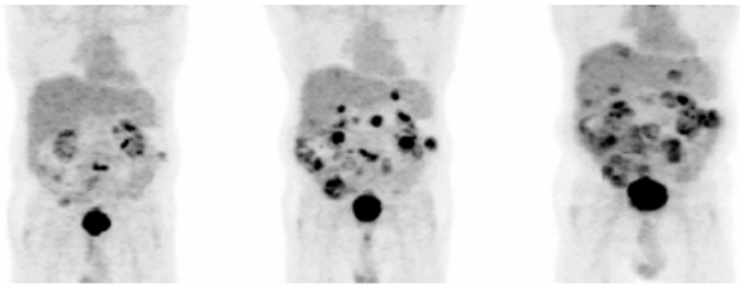
PET Scanner

Glucose Metablism (FDG)



Baseline 24 hrs 7 days 2 mos 5.5 mos

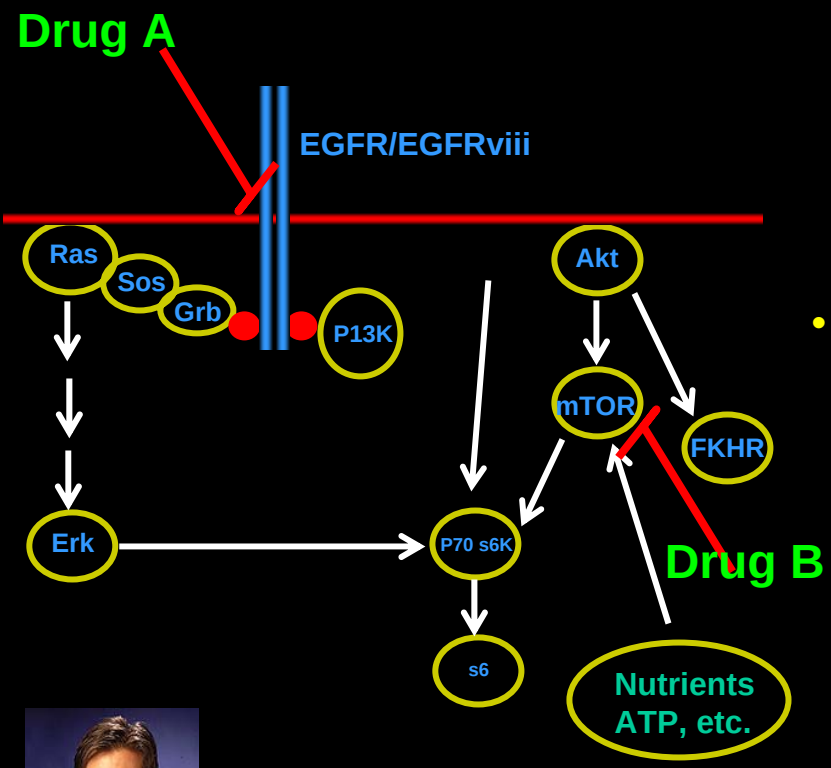
Positive Response



Baseline 1 month 2 months

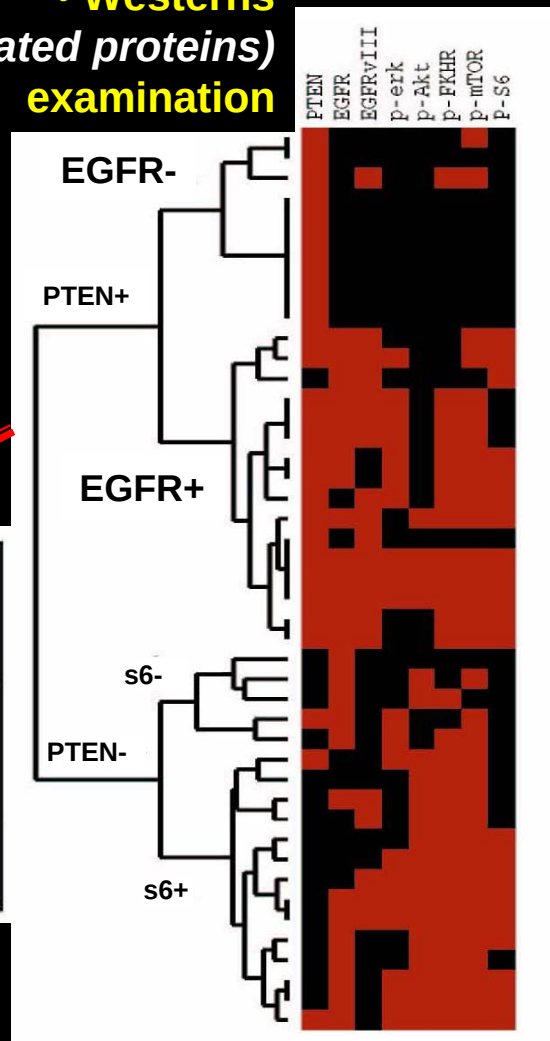
Refractory

Cancer Pathways: Knowledge ripe for clinical applications



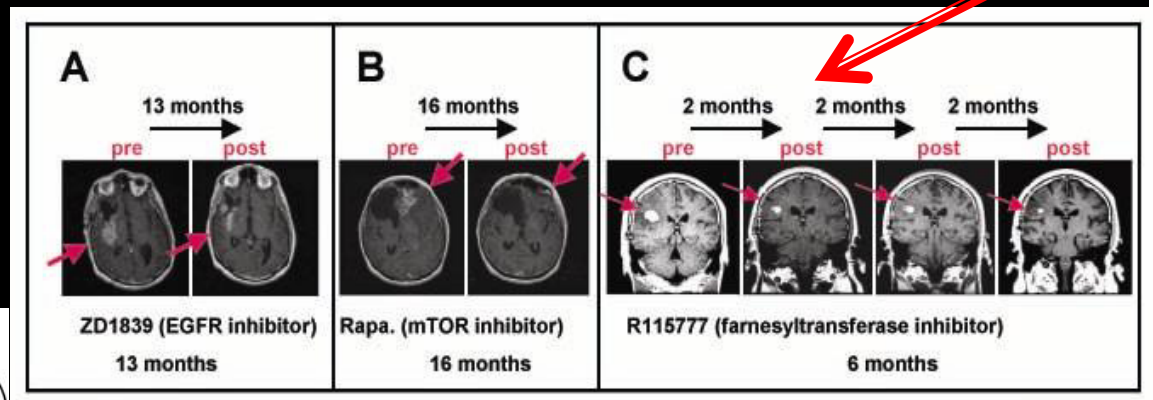
Pathology TREE COMBINES

- cDNA's
- Westerns
- IHC staining (*phosphorylated proteins*)
- phenomenological examination



To produce a positive outcome for patients

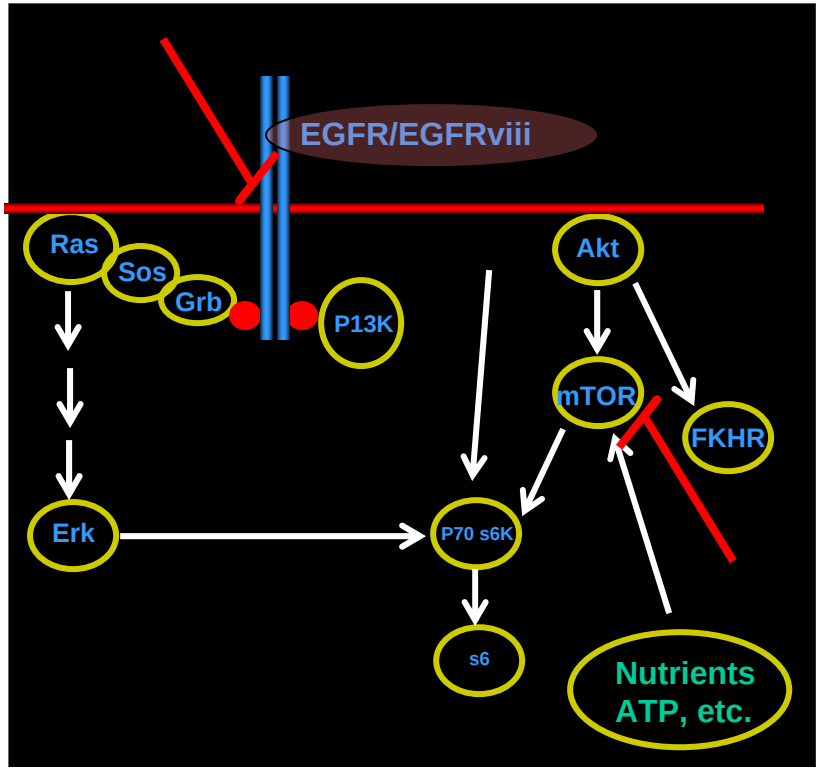
Paul Mischel



NANOSYSTEMS BIOLOGY N S B C C CANCER CENTER

UCLA

Limitations of Cancer Pathways Concept



EGFR is implicated in the following diseases

Asthma Bone Neoplasms Breast Neoplasms Carcinoma, Ductal, Breast Carcinoma, Hepatocellular Carcinoma, Non-Small-Cell Lung Carcinoma, Pancreatic Ductal Carcinoma, Small Cell Carcinoma, Squamous Cell Cervix Neoplasms Colonic Neoplasms Colorectal Neoplasms Endometrial Neoplasms Esophageal Neoplasms Gastrointestinal Neoplasms Glioma Head and Neck Neoplasms Lung Neoplasms Melanoma Multiple Myeloma Ovarian Neoplasms Pancreatic Neoplasms Prostatic Hyperplasia Prostatic Neoplasms Psoriasis Skin Neoplasms Uterine Neoplasms

~20% of the proteins that are known correlates of Ovarian Cancer

•increased serine phosphorylation of AKT1 correlates with serous cystadenocarcinoma associated with ovarian neoplasms increased abnormal cleavage of B4GALT1 may correlate with ovarian neoplasms (1384956)
•increased phosphorylation of BAD may correlate with drug-resistant form of ovarian neoplasms (10769688)
•increased cleavage of BAX may correlate with increased response to drug associated with ovarian neoplasms (12085231)
•decreased cleavage of BCL2 may correlate with increased apoptosis associated with ovarian neoplasms (12085231)
•increased cleavage of CASP8 may correlate with increased response to drug associated with ovarian neoplasms (12907626, increased processing of CASP8 may correlate with increased response to drug associated with ovarian neoplasms (11859411, increased cleavage of CD44 correlates with carcinoma tumors associated with ovarian neoplasms (11839564)
•tyrosine phosphorylation of CTTN may cause increased cell migration associated with ovarian neoplasms (11084024)
•increased proteolysis of DCN may correlate with ovarian neoplasms (12060613)
•increased phosphorylation of EGFR may cause increased cell proliferation associated with ovarian neoplasms (10702246)
•decreased phosphorylation of ERBB2 may cause increased apoptosis associated with ovarian neoplasms (11724334)
•increased phosphorylation of ETS2 may correlate with increased Ras protein signal transduction associated with ovarian neoplasms increased phosphorylation of ETV1 may correlate with increased negative regulation of transforming growth factor beta receptor signaling increased secretion of FASLG may cause decreased T-lymphocytes survival associated with ovarian neoplasms (14500397)
•increased secretion of FBLN1 may correlate with malignant form of ovarian neoplasms (8552629)
•increased tyrosine phosphorylation of GJA1 may cause decreased cell-cell signaling associated with ovarian neoplasms (12907686)
•increased glycosylation of HP correlates with ovarian neoplasms (1451094)
•decreased secretion of IL1A may correlate with carcinoma tumors associated with ovarian neoplasms (9061376)
•increased secretion of IL8 may correlate with increased response to hypoxia associated with ovarian neoplasms (10582705)
•increased phosphorylation of JUN may correlate with drug-sensitive form of ovarian neoplasms (12637505)
•increased secretion of KLK5 correlates with carcinoma tumors associated with ovarian neoplasms (12738725)
•decreased processing of MAP3K1 correlates with drug-resistant form of ovarian neoplasms (10639576)
•abnormal phosphorylation of MAP3K4 may correlate with increased anti-apoptosis associated with ovarian neoplasms (10938285)
•increased phosphorylation of MAP3K5 may correlate with decreased response to drug associated with ovarian neoplasms increased processing of MMP2 may correlate with ovarian neoplasms (10207247)
•increased cleavage of MT2A may cause increased apoptosis associated with ovarian neoplasms (11807957)
•abnormal secretion of PLAU correlates with decreased occurrence of disease progression associated with ovarian neoplasms increased cleavage of PLAUR correlates with ovarian neoplasms (8699658)
•decreased phosphorylation of RB1 may correlate with increased response to drug associated with ovarian neoplasms (11753676)
•increased secretion of SERPINE1 correlates with increased incidence of less severe form of ovarian neoplasms (8826920)
•decreased phosphorylation of STAT3 may prevent increased cell proliferation associated with ovarian neoplasms (11753675)
•decreased secretion of TGFA may correlate with decreased cell migration associated with ovarian neoplasms (11350918)
•decreased secretion of TIMP1 may correlate with invasive form of ovarian neoplasms (11719460)
•increased secretion of TIMP2 may correlate with increased response to drug associated with ovarian neoplasms (9174131)
•decreased secretion of TNF may correlate with carcinoma tumors associated with ovarian neoplasms (9061376)
•increased phosphorylation of TYK2 may correlate with ovarian neoplasms (9009165)

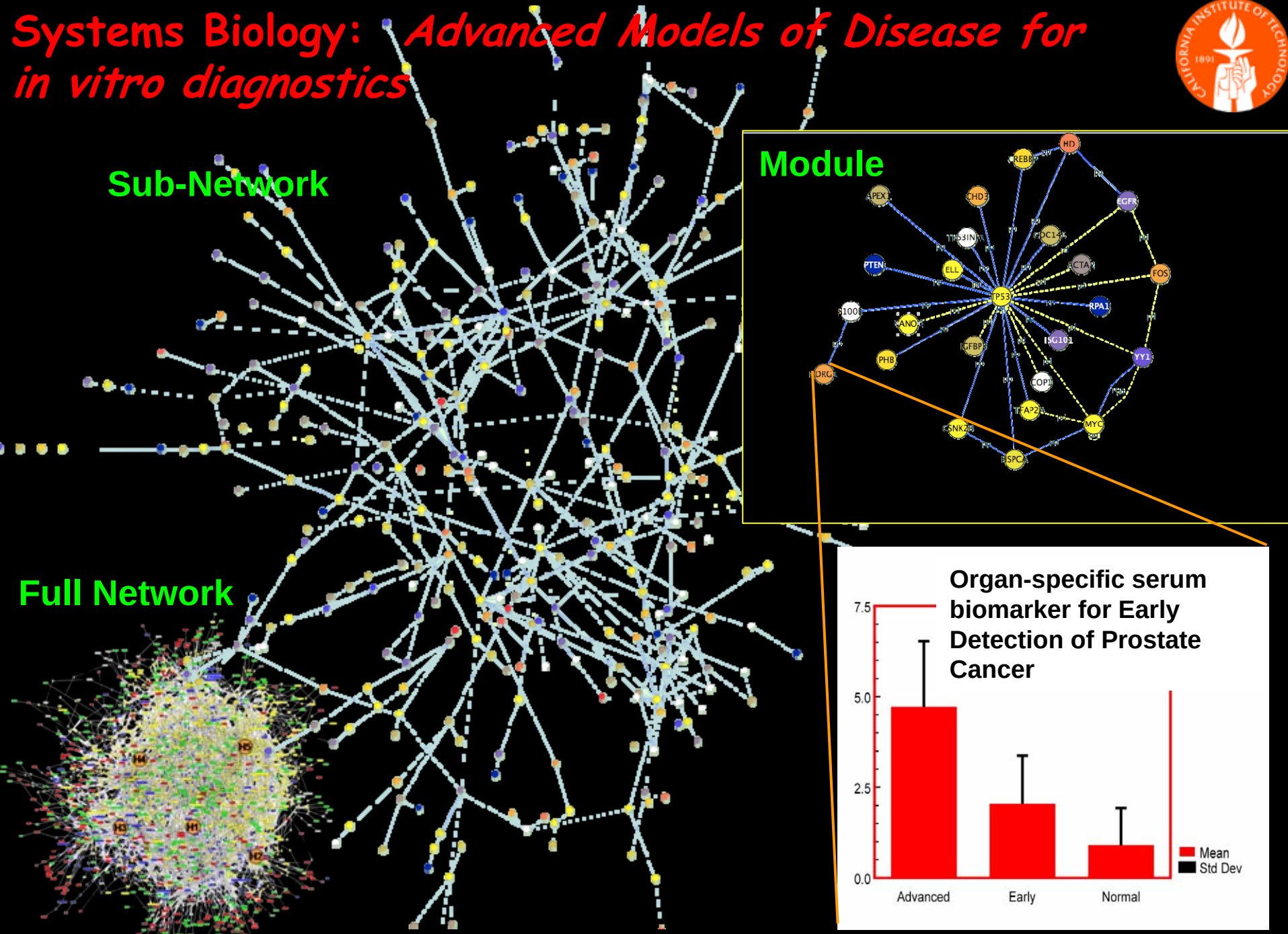
increased expression of ABCC2 protein may cause decreased response to drug associated with ovarian neoplasms differential expression of ACPP protein correlates with carcinoma tumors associated with ovarian neoplasms (8348500)
increased expression of AKT1 protein correlates with ovarian neoplasms (10822383)
increased expression of AMBP protein does not correlate with increased cell migration associated with ovarian neoplasms
increased expression of ANGPT1 protein correlates with adenocarcinoma tumors associated with ovarian neoplasms
decreased expression of B2M protein correlates with adenocarcinoma tumors associated with ovarian neoplasms (11989974)
increased expression of BAD protein may cause increased occurrence of drug-sensitive form of ovarian neoplasms (9824152)
decreased expression of BARX2 protein correlates with clear cell adenocarcinoma associated with ovarian neoplasms
increased expression of BAX protein may correlate with increased response to drug associated with ovarian neoplasms
increased expression of BCL2L1 protein correlates with drug-resistant form of ovarian neoplasms (10639576)
decreased expression of BIRC4 protein may prevent increased inhibition of caspase activation associated with ovarian
increased expression of BIRC5 protein correlates with advanced stage form of ovarian neoplasms (12118327)
increased expression of BSG protein correlates with increased occurrence of death associated with ovarian neoplasms
increased expression of BZRP protein correlates with ovarian neoplasms (9592189)
increased expression of CASP3 protein may prevent increased occurrence of death associated with ovarian neoplasms
decreased expression of CAV1 protein correlates with carcinoma tumors associated with ovarian neoplasms (11032026)
increased expression of CCL2 protein correlates with cystadenocarcinoma associated with ovarian neoplasms (11978786)
increased expression of CCL22 protein correlates with carcinoma tumors associated with ovarian neoplasms (15322536)
abnormal expression of CCND1 protein may correlate with abnormal estrogen receptor signaling pathway associated with
increased expression of CD24 protein correlates with increased severity of carcinoma associated with ovarian neoplasms
abnormal expression of CD58 protein may not correlate with carcinoma associated with ovarian neoplasms (8985099)
increased expression of CD68 protein correlates with decreased cell differentiation associated with ovarian neoplasms
increased expression of CD99 protein may correlate with peripheral primitive neuroectodermal tumors associated with ovarian
increased expression of CDC25A protein may correlate with more severe form of ovarian neoplasms (11205229)
differential expression of CDH2 protein correlates with ovarian neoplasms (9191009)

decreased expression of FGF2 protein correlates with increased response to drug associated with ovarian
increased expression of FGF7 protein may correlate with increased response to hormone stimulus associated
increased expression of FGF8 protein correlates with ovarian neoplasms (11072239)
lack of expression of FHIT protein correlates with more severe form of ovarian neoplasms (11461085)
increased expression of FLT1 protein may prevent increased incidence of death associated with ovarian increased
expression of FLT4 protein correlates with neoplasm metastasis associated with ovarian neoplasms decreased
expression of FOLR1 protein may correlate with decreased cell proliferation associated with ovarian increased
expression of FOS protein may correlate with increased response to acetate associated with ovarian decreased
expression of FPGS protein may correlate with ovarian neoplasms (12084463)
increased expression of FUT8 protein correlates with increased N-linked glycosylation associated with ovarian
increased expression of GAS protein correlates with increased incidence of malignant form of ovarian lack of
expression of GATA6 protein may cause abnormal cell differentiation associated with ovarian neoplasms differential
expression of GFAP protein correlates with ovarian neoplasms (7821917)
decreased expression of GJA1 protein correlates with serous cystadenocarcinoma associated with ovarian
increased expression of GNRH1 protein may prevent increased cell proliferation associated with ovarian increased
expression of GNRH2 protein may cause decreased cell proliferation associated with ovarian increased expression
of GSTP1 protein correlates with decreased response to drug associated with ovarian increased expression of HGF
protein causes increased apoptosis associated with ovarian neoplasms increased expression of HOXB7 protein
correlates with carcinoma tumors associated with ovarian neoplasms
decreased expression of HRASLS protein correlates with carcinoma tumors associated with ovarian neoplasms
decreased expression of ICAM1 protein correlates with increased incidence of death associated with ovarian
increased expression of IGF1R protein correlates with carcinoma tumors associated with ovarian neoplasms
increased expression of IGF2 protein may cause ovarian neoplasms
decreased expression of IGFBP3 protein correlates with advanced stage form of ovarian neoplasms increased
expression of IL10 protein may prevent increased cell proliferation associated with ovarian neoplasms increased
expression of IL6 protein correlates with carcinoma tumors associated with ovarian neoplasms increased
expression of IL8 protein may correlate with increased response to hypoxia associated with ovarian abnormal
expression of INSR protein may cause abnormal insulin receptor signaling pathway associated with increased
expression of IRF1 protein may correlate with increased response to drug associated with ovarian decreased
expression of ITGA6 protein may correlate with decreased cell migration associated with ovarian increased
expression of ITGAV protein may correlate with abnormal cell-matrix adhesion associated with decreased
expression of ITGB3 protein correlates with ovarian neoplasms (8476198)
increased expression of ITGB4 protein may cause increased cell proliferation associated with ovarian increased
expression of ITGB6 protein correlates with advanced stage form of ovarian neoplasms (11872628)
decreased expression of JUN protein may correlate with decreased severity of carcinoma associated with
increased expression of KIT protein correlates with carcinoma associated with ovarian neoplasms (12910520)

increased expression of COL18A1 protein does not cause increased negative regulation of angiogenesis associated increased
expression of COPS5 protein correlates with benign form of ovarian neoplasms (11751512)
decreased expression of CRYAB protein correlates with more severe form of ovarian neoplasms (14695176)
increased expression of CSF1 protein correlates with increased occurrence of death associated with ovarian decreased
expression of CTNNB1 protein correlates with serous cystadenocarcinoma associated with ovarian increased expression of
CTSL2 protein correlates with carcinoma tumors associated with ovarian neoplasms decreased expression of CXADR protein
correlates with more severe form of ovarian neoplasms (12140737)
increased expression of CXCL12 protein may correlate with neoplasm metastasis associated with ovarian neoplasms
increased expression of CYP1B1 protein correlates with malignant form of ovarian neoplasms (11461084)
lack of expression of DAB2 protein correlates with abnormal cellular morphogenesis associated with ovarian lack of expression
of DCK protein may cause decreased response to drug associated with ovarian neoplasms (8033147, increased expression of
DCN protein may prevent increased cell proliferation associated with ovarian neoplasms increased expression of DIABLO
protein may prevent decreased apoptosis associated with ovarian neoplasms lack of expression of DIRAS3 protein may cause
decreased negative regulation of cell proliferation associated with ovarian decreased expression of DPH2L1 protein may cause
increased cell proliferation associated with ovarian neoplasms increased expression of DUSP1 protein may prevent increased
cell proliferation associated with ovarian neoplasms increased expression of EDG2 protein may cause increased apoptosis
associated with ovarian neoplasms increased expression of EDG4 protein may cause more severe form of ovarian neoplasms
increased expression of EDG7 protein may correlate with increased cell proliferation associated with ovarian increased
expression of EDN1 protein may cause abnormal signal transduction associated with ovarian neoplasms increased expression
of EDN1 protein may cause pathologic neovascularization associated with ovarian neoplasms increased expression of EEF2
protein correlates with ovarian neoplasms

increased cytosol localization of CAV1 correlates with less severe form of ovarian neoplasms (11696424)
abnormal extracellular space localization of CCL2 correlates with carcinoma tumors associated with ovarian neoplasms
increased nucleus localization of CTNNB1 correlates with endometrioid carcinoma associated with ovarian neop
mislocalization of GATA6 protein correlates with carcinoma associated with ovarian neoplasms (12941822)
increased extracellular space localization of ICAM1 correlates with disease progression associated with ovari
absence of nucleoplasm localization of MPG correlates with mucinous cystadenocarcinoma associated with
increased cytosol localization of PLAU correlates with increased occurrence of disease progression associat
increased nucleus localization of TFAP2A correlates with increased occurrence of death associated with ovari

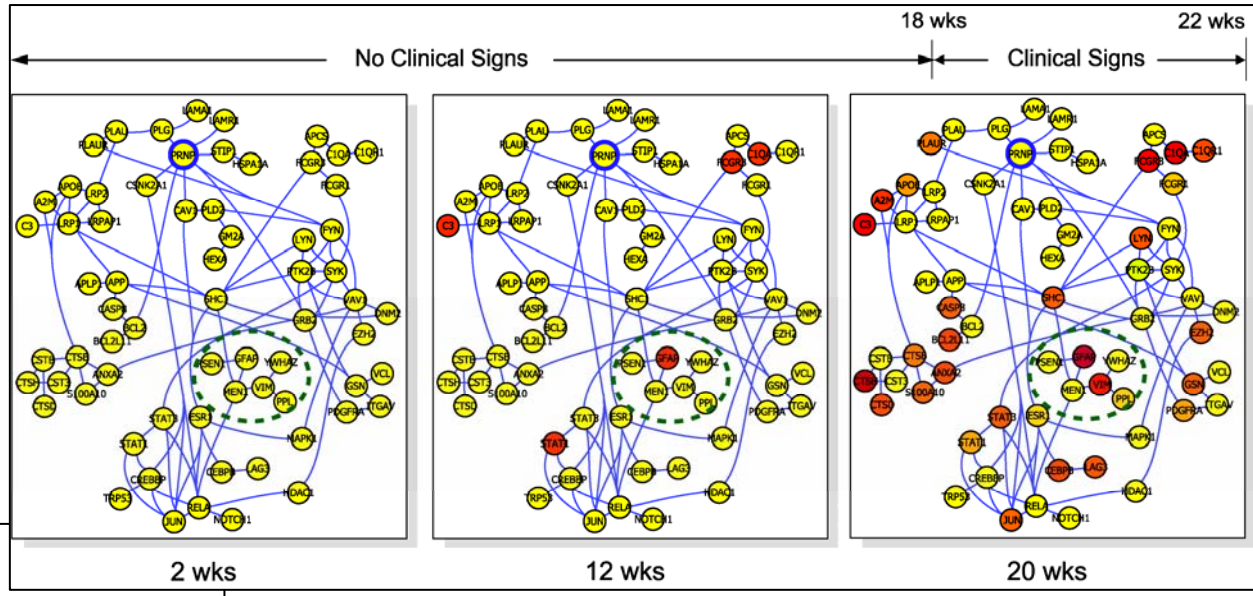
Systems Biology: *Advanced Models of Disease for in vitro diagnostics*

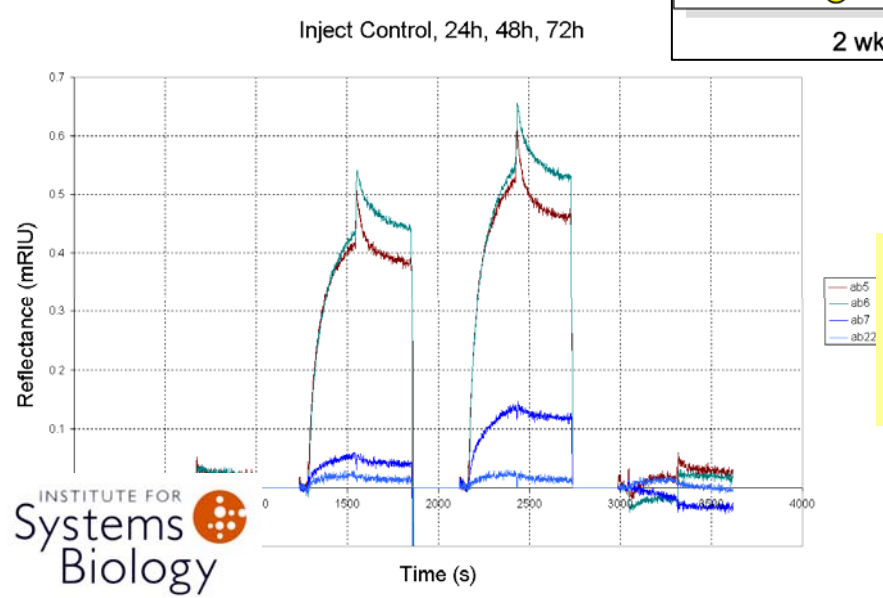


Biaoyang Lin, Inst. for Systems Biology (unpublished, 2007)

Increasing the Signal to Noise in Biological Measurements

Dynamic Network Models





Evolution of liver-specific proteins during the course of a toxic event

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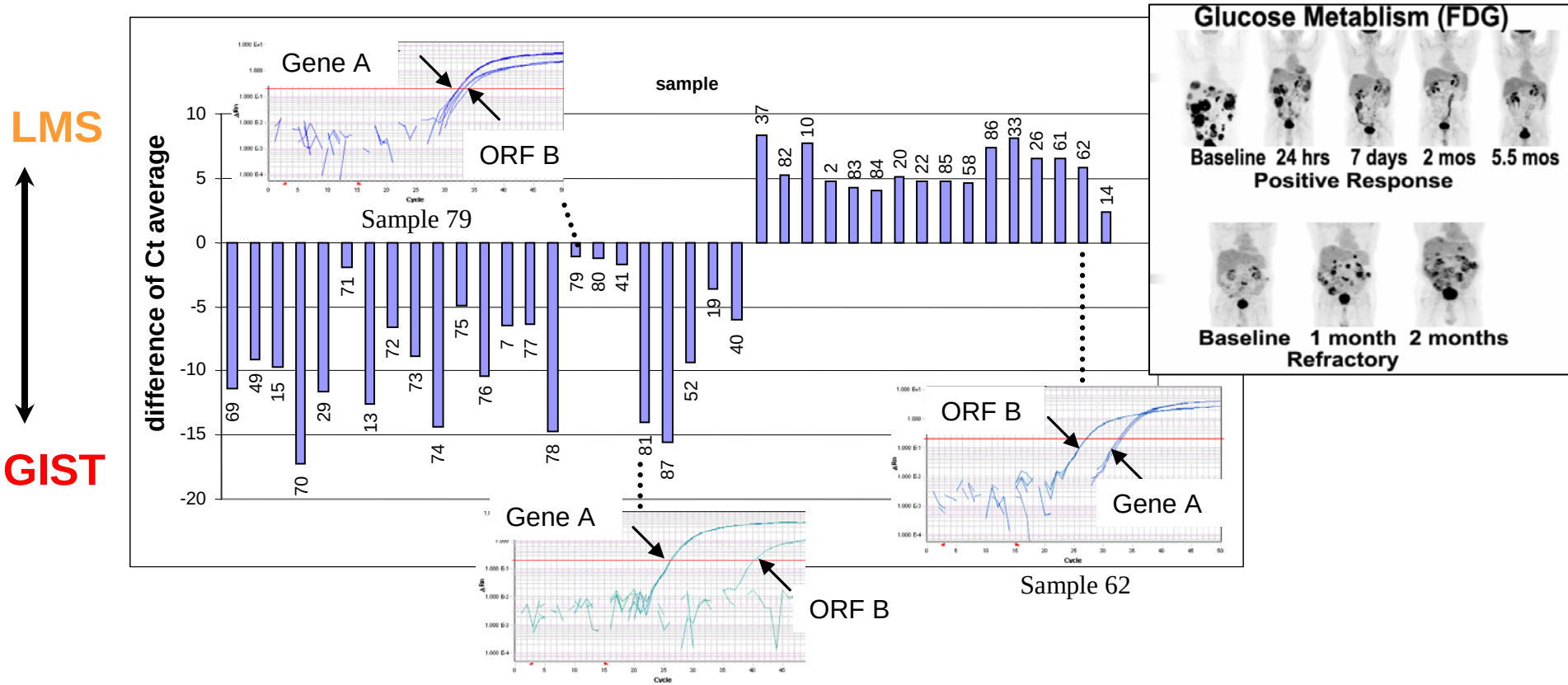
NanoMedicine: Systems Biology guided in vitro Diagnostics

100% differentiation between phenotypically identical tumors: GIST & LMS

Test in clinical use at MD Anderson and elsewhere



GIST Patients: Gleevec responsive; , doxorubicin non-responsive

LMS Patients: Gleevec non-responsive; , doxorubicin responsive

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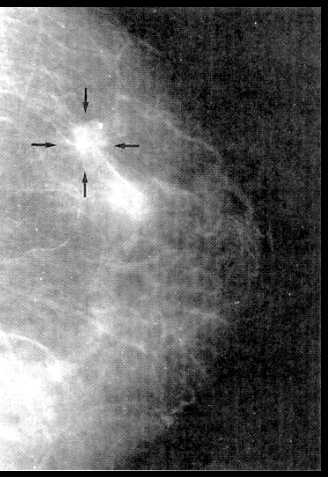
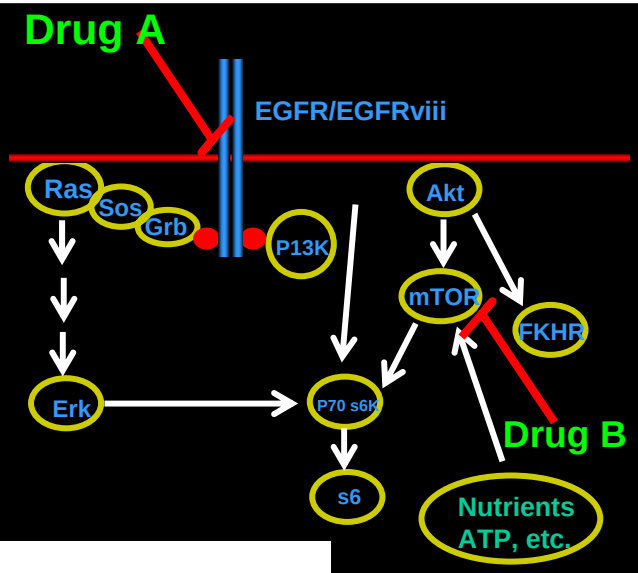
In principle, we can answer these questions through blood measurements

- Do I have cancer?
- Which organ is it in?
- Early stage, late stage, metastatic?
- Appropriate therapy or combination therapies?
- If I'm on therapy: What are the positive and adverse responses?
- Best diet practices? Best exercise practices?

Clinical diagnostics that can follow the route:

Pauciparameter → pathways → networks





MUST BE CHEAP

A South Pointing Chariot
(an early Chinese invention)

Lodestone floating on water
(another early Chinese invention)

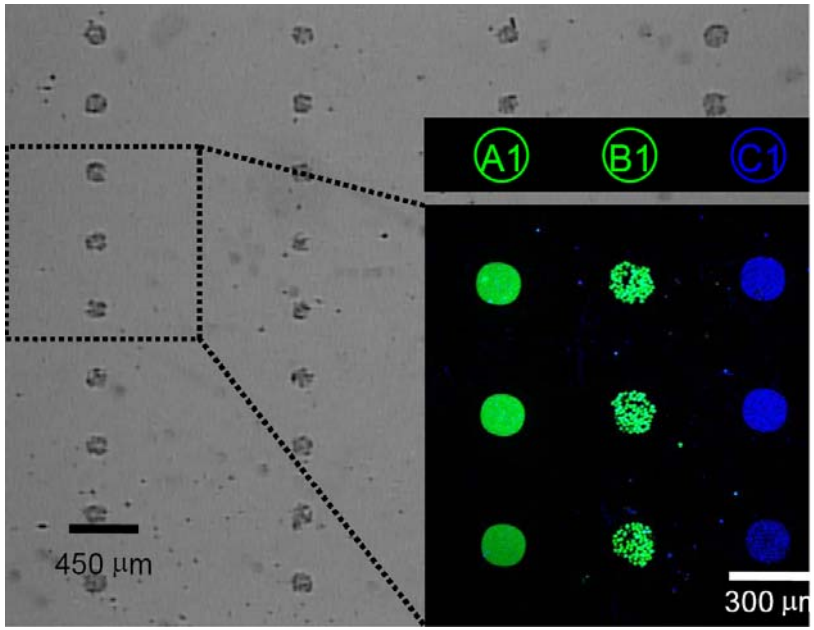
In vitro Measurement Technologies

DEAL

Glass, plastic & reagents

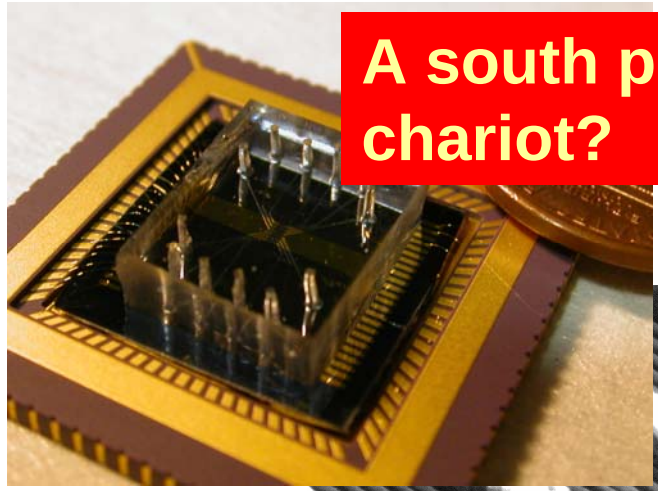
- Rapid
- Sensitive
- Inexpensive
- Multiparameter & Scalable
- Quantitative

cDNAs & Proteins & Cells co-Detected

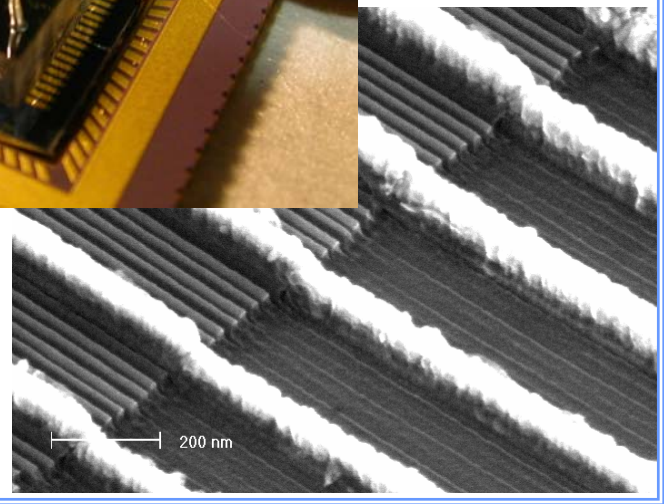


Nanowires

- Rapid
- Sensitive
- Implantable or Wearable
- Label-free & Quantitative



A south pointing chariot?



Making Measurements Cheap

- **GET THE BIOLOGY RIGHT**
- **glass & plastic**; routine, scalable fabrication
- **inexpensive reagents** – KEY PROBLEM!!
- **small amounts of tissue or blood**

  (fingerpricks or skinny needle biopsies)
- **rapid, sensitive, multiparameter measurements**
- **simple measurement instrumentation**
- **minimal human intervention**

Nanotech involved in the solution

Measurements must be sensitive, cover a broad concentration range, taken from tissues, blood, etc.

Conventional Blood protein measurement

Extract ~1 ml blood

large sample size

Centrifuge to separate plasma or serum from whole blood

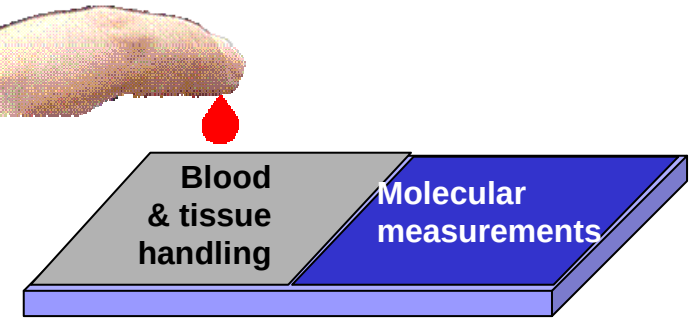
human intervention – costly & time consuming

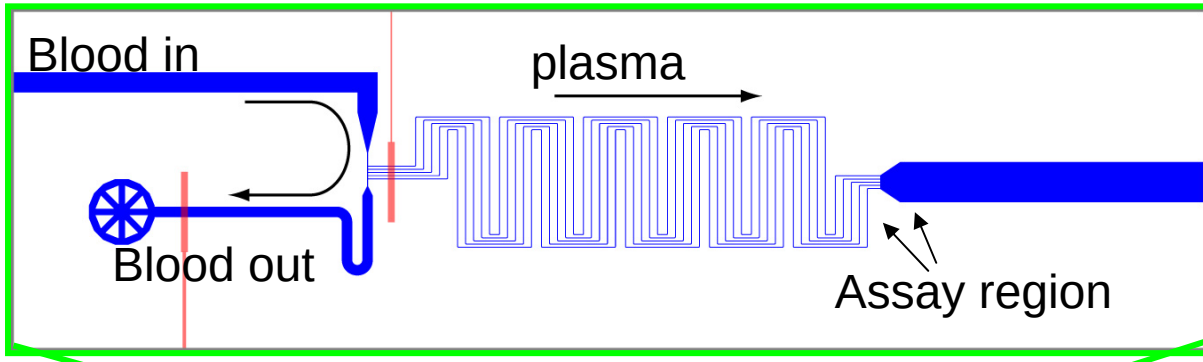
Measure proteins in 96 well plate using ELISA

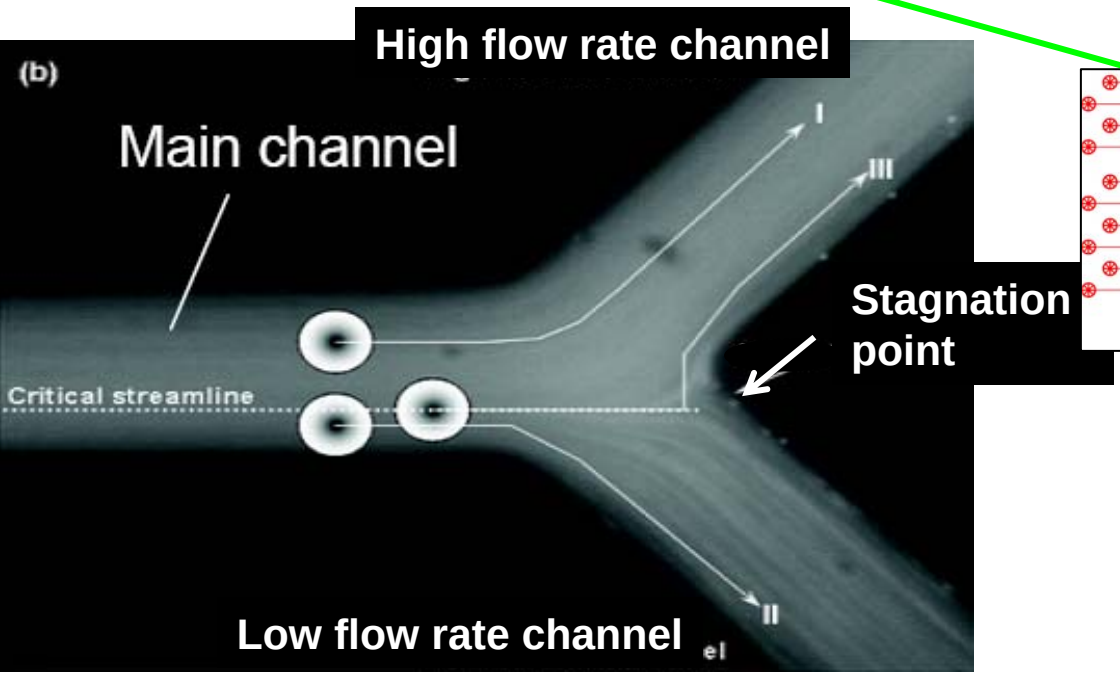
time consuming, not particularly sensitive, expensive reagents, hard to be quantitative, etc.

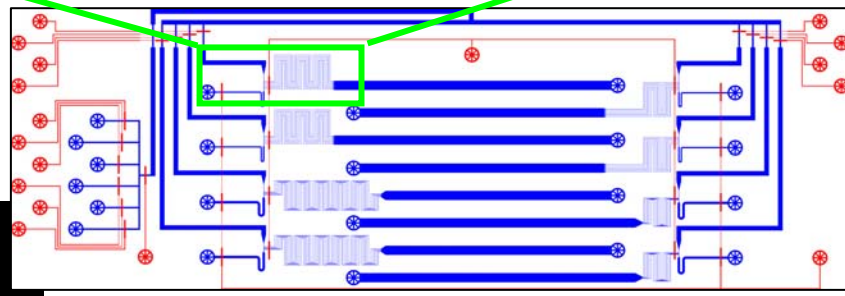
Measure gene expression (mRNAs) using PCR and completely separate instrumention & protocols

Rapid, on-chip separation of plasma from whole blood







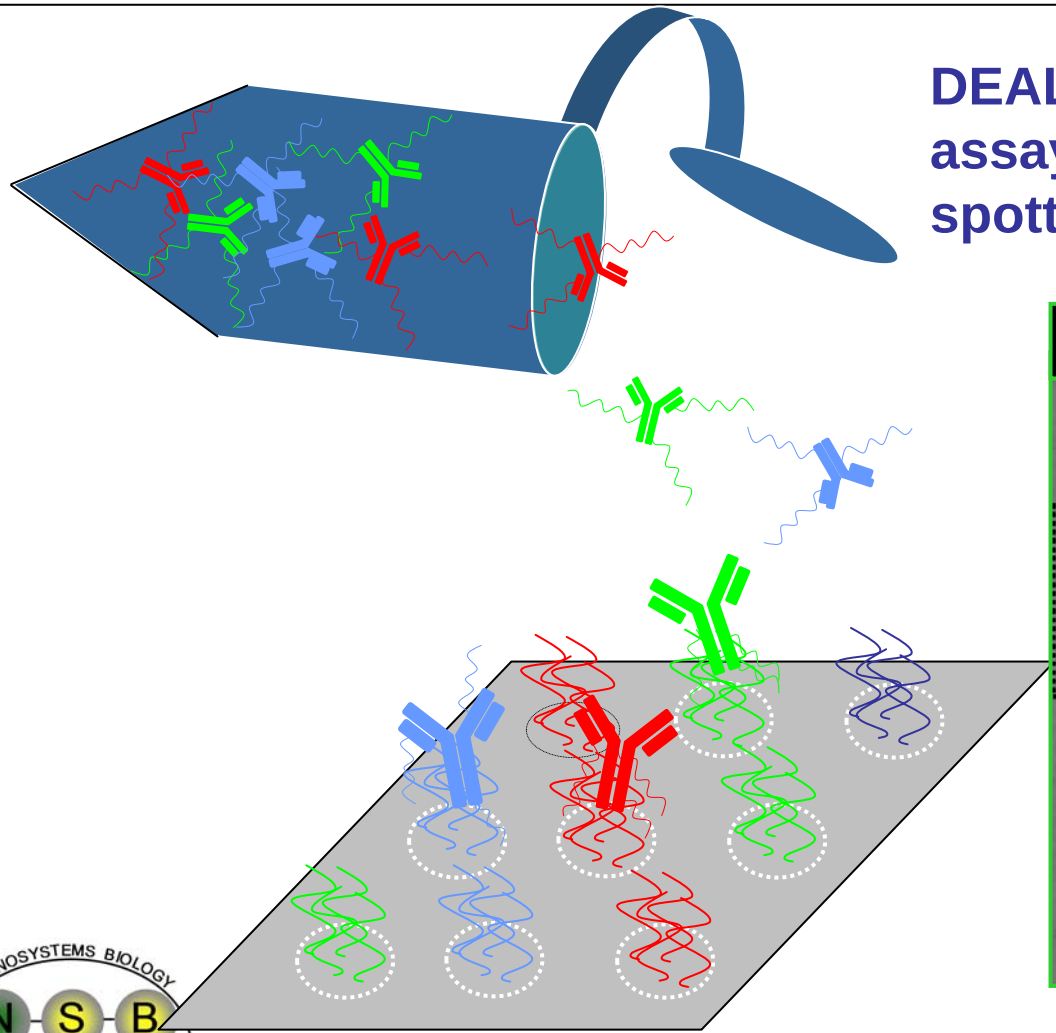


5-20 cents per protein measurement if whole chip costs $50

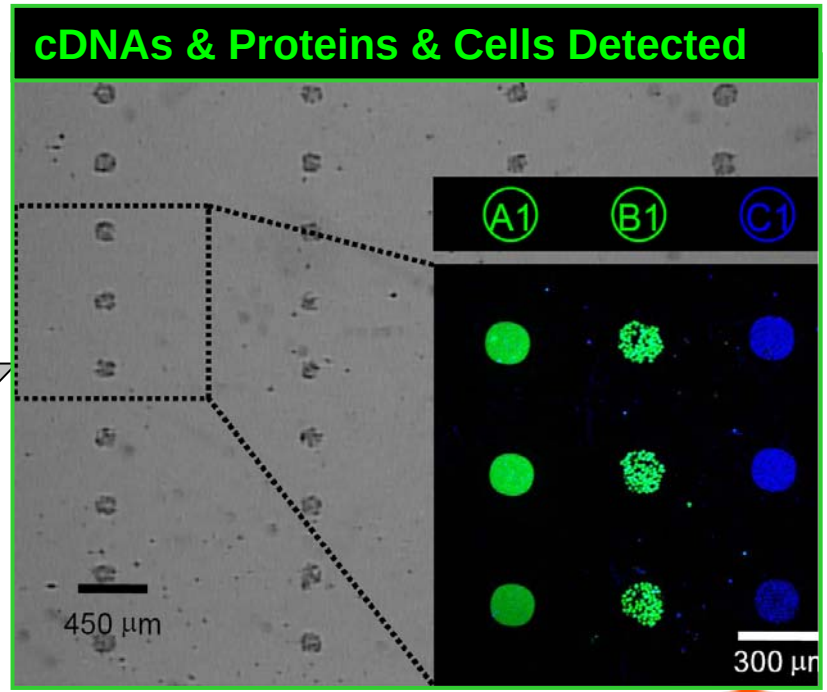
Yang, *et. al*. 2006. *Lab Chip*, **6**: 871-880.

DEAL:DNA-encoded antibody libraries for multiparameter diagnostics

- **co-detection of cDNAs, proteins, and cells**



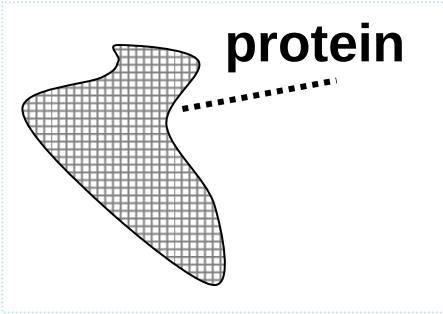
DEAL encoded protein sandwich assays self-assemble onto a DNA spotted array

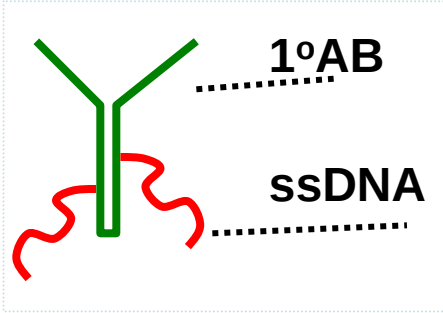


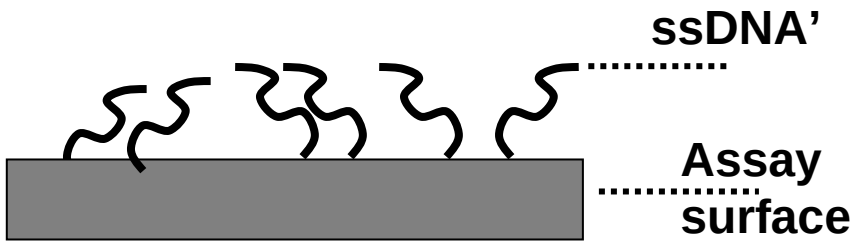
R.Bailey, et al., JACS 2007

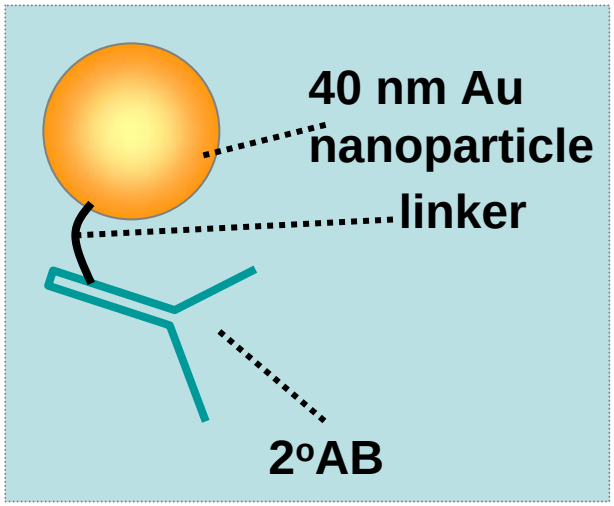
DIGITAL DEAL

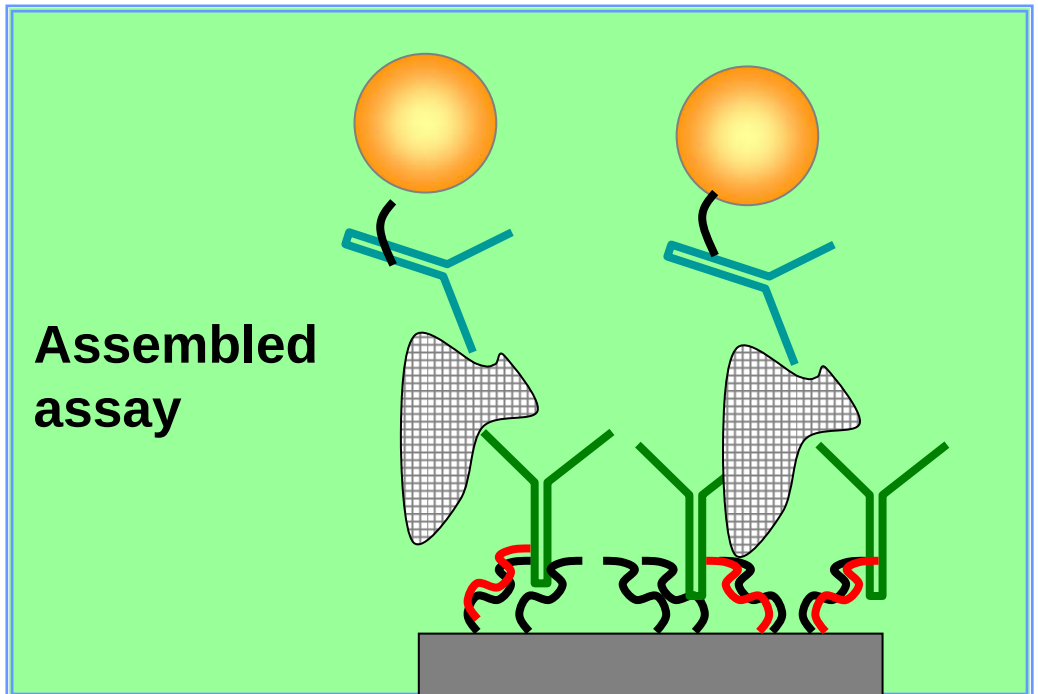
- Sensitivity to 10^{-16}M (100 attoM)
- Concentration Range to ~10^{6}
- Quantitative (no calibration)
- automated, digital analysis





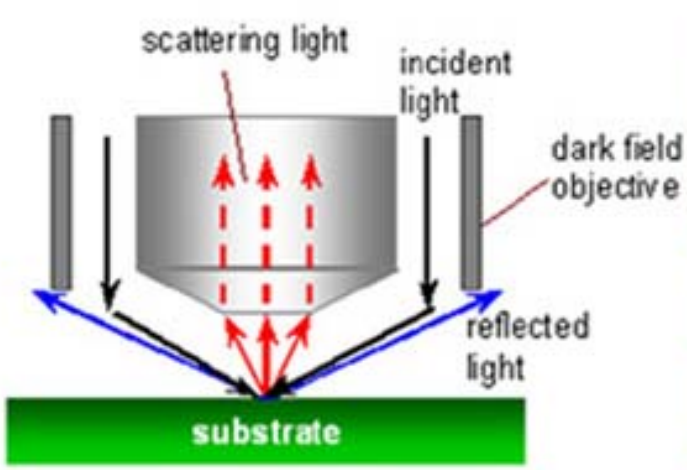


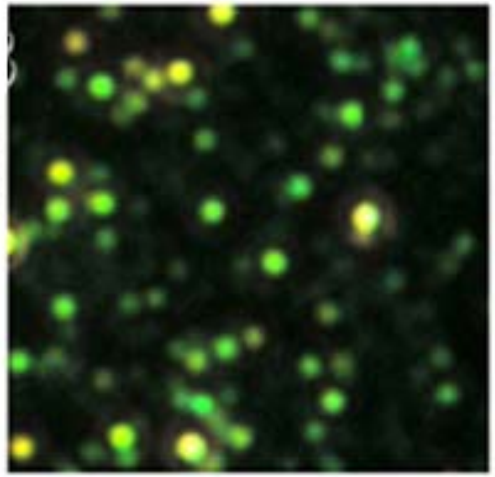




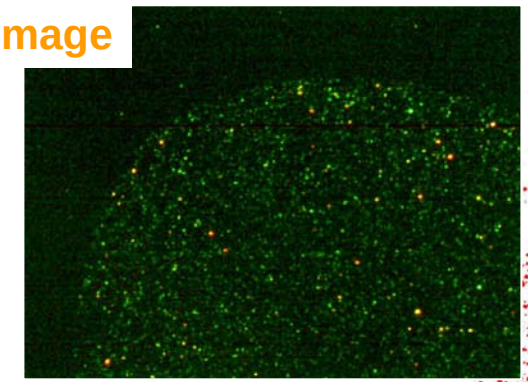
Digital DEAL Proteomics for serum analysis

Image with a dark-field light scattering microscope (can be molded plastic)

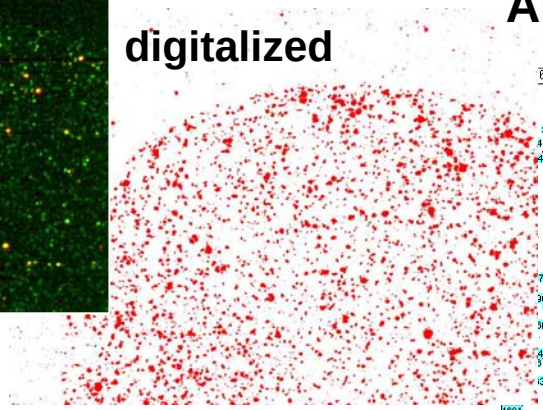




df image

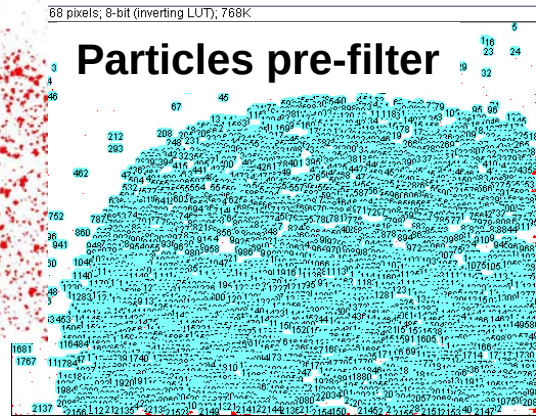


digitalized

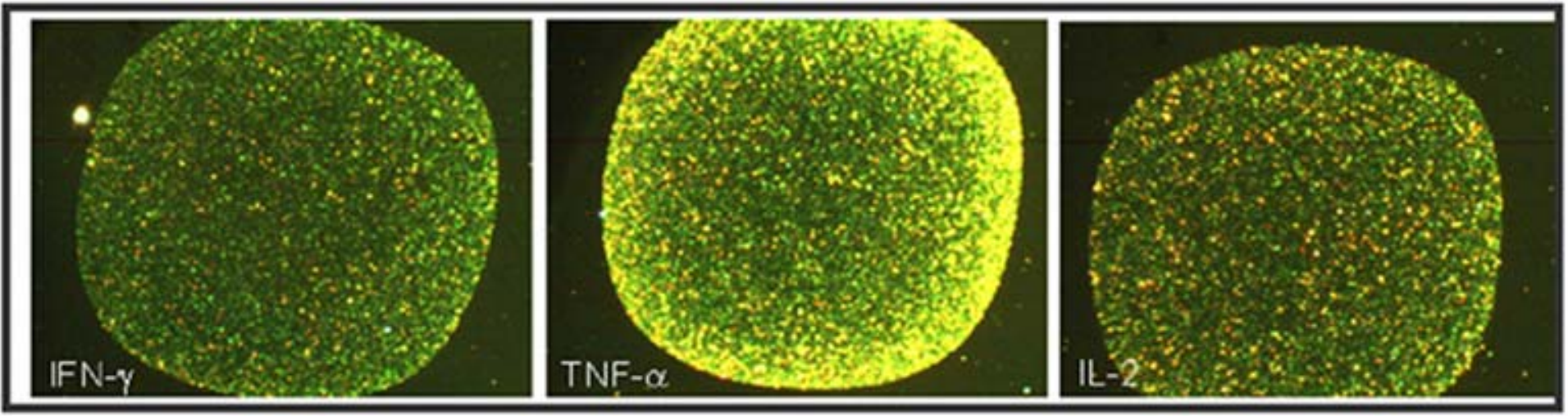


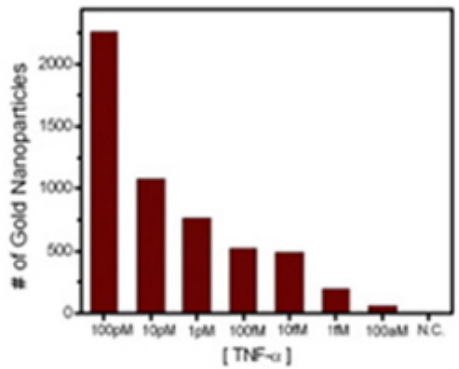
Automated particle counting

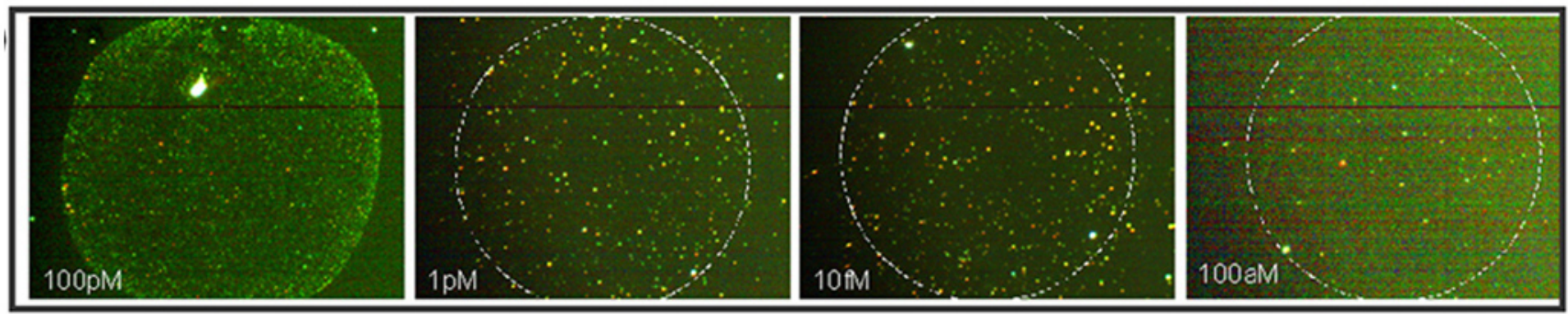
Particles pre-filter



Digital Proteomics



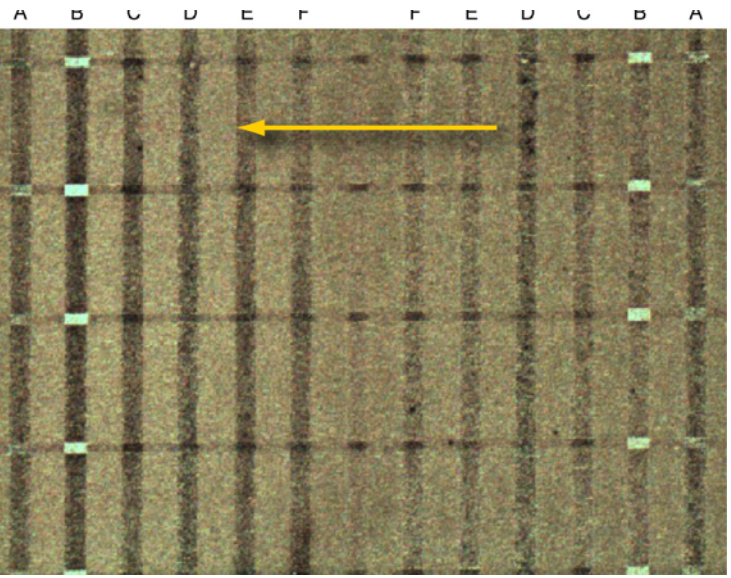




Assays from whole blood separated on chip

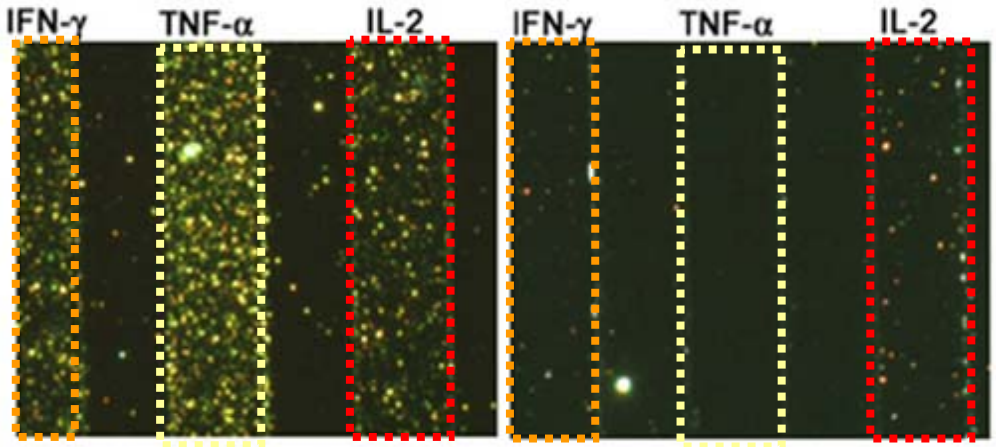
(2 proteins spiked in to whole blood)

5 minute assay (plasma separated from blood on chip)
(5 measurements per protein)



Spiked blood (10 pM)

Healthy blood

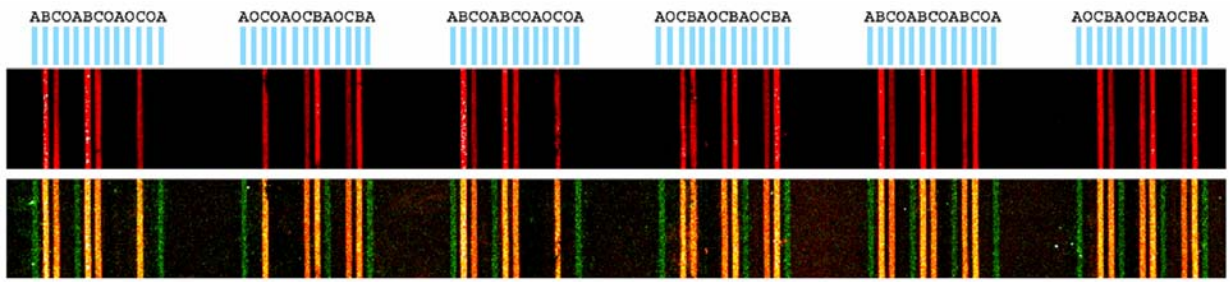


~1 femtoM

<100 attoM

~5 femtoM

DEAL Barcode-type Assay



What is nanotech giving to *in vitro* diagnostics?

Previous: concentration range detected: 10^2-10^3

Now: 10^4-10^6

better match to range of relevant proteins

Previous: protein detection limit ~10^{-12} M

Now: ~10^{-16} M

key disease biomarkers now detectable

Previous: proteins & mRNAs detected from ~10^4 cells

Now: multiple measurements from single cells

heterogeneity of diseased tissues

analysis of stem cells and circulating tumor cells

Potentially significant savings in reagents, time for measurements, assay costs, tissue requirements, etc.

Challenges for *in vitro* diagnostics?

To continue to scale protein measurements ***(i.e. doubling number of protein measurements from tissues every year at constant cost)***

- ***Surface science***
- ***different proteins have different expected concentration ranges – integrate onto one platform***
- ***Computation methods for integrating measurements into a simple diagnosis***
- ***Protein capture agents!!!***
- ***Tissue handling protocols***
- ***Biology***
- ***Integration of biology, chemistry & fabrication remains a key challenge***
- ***Integration of mRNA and protein measurements remains a challenge***