BIOE 301

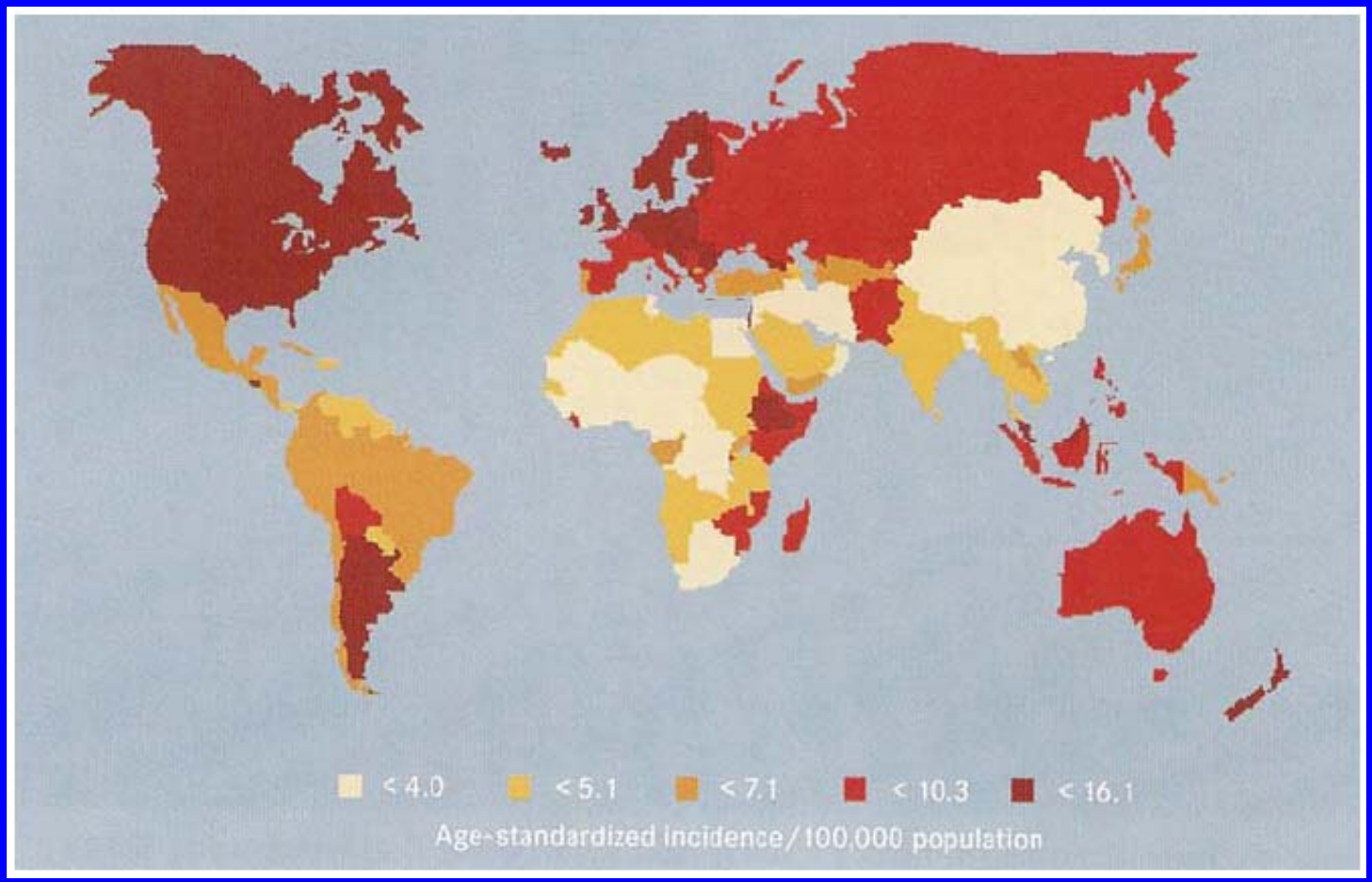
Lecture Fifteen

Bioengineering and Ovarian Cancer

Statistics on Ovarian Cancer

- United States:
  - Incidence: 22,430
  - Mortality: 15,280
- Worldwide:
  - Incidence: 190,000
  - Mortality: 114,000

Global Burden of Ovarian Cancer

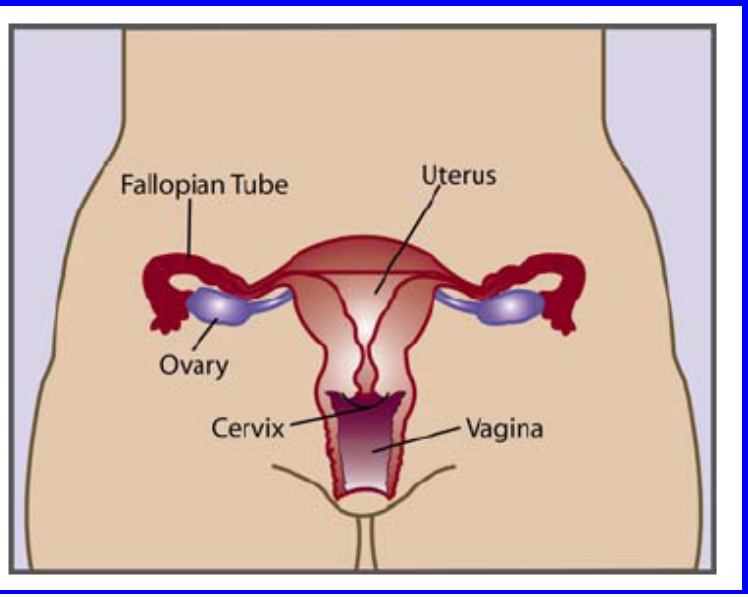


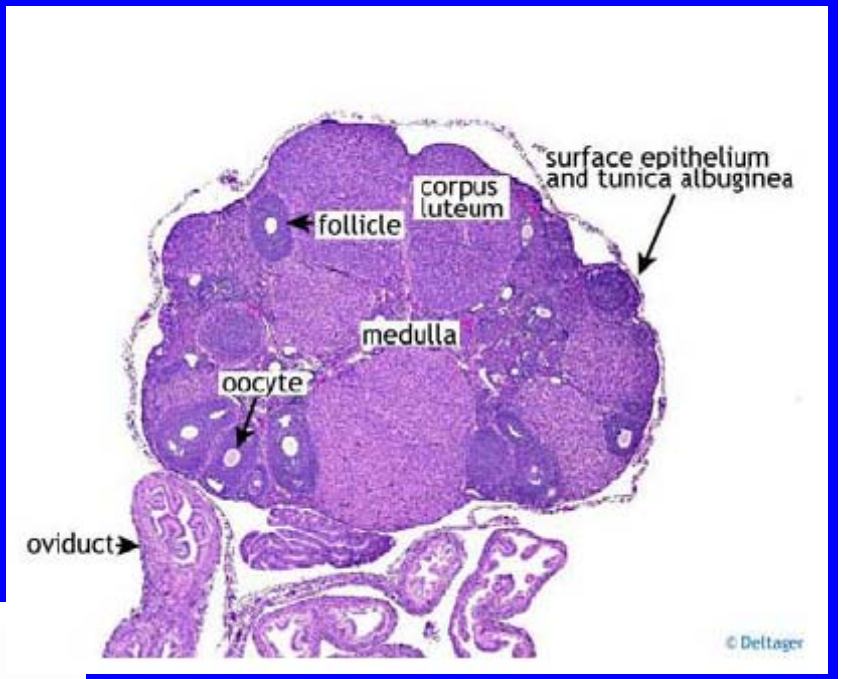
Risk factors

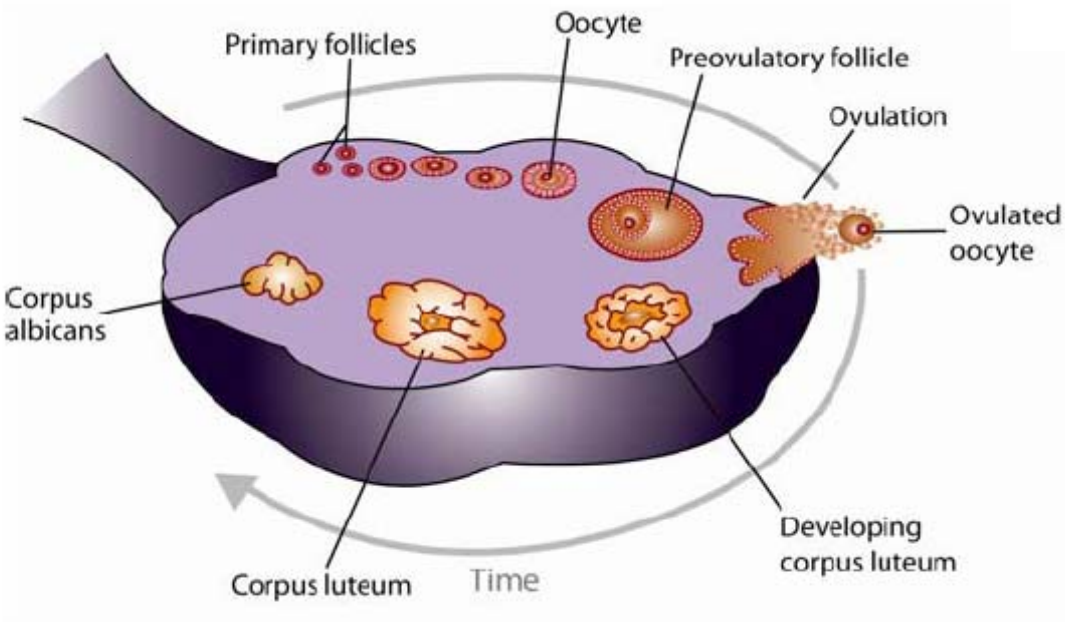
- Age
  - Most ovarian cancers develop after menopause
- Personal or family history of breast, ovarian, endometrial, prostate or colon cancer.
- Reproductive history

  Increases with the more lifetime cycles of ovulation that a woman has undergone. Thus, women who have undergone hormonal treatment for infertility, never used birth control pills, and who never became pregnant are at higher risk for ovarian cancer

Pathophysiology



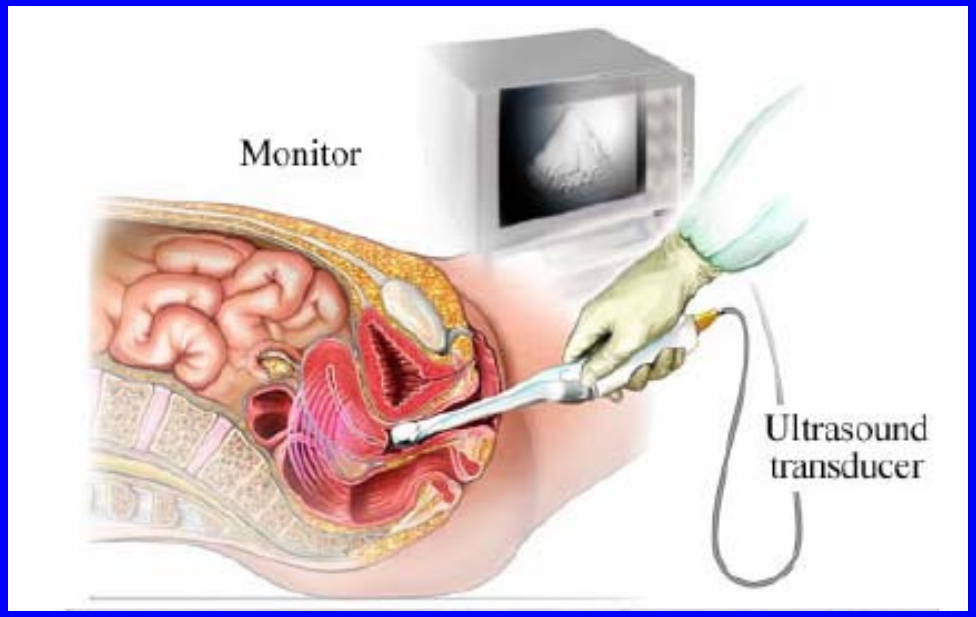


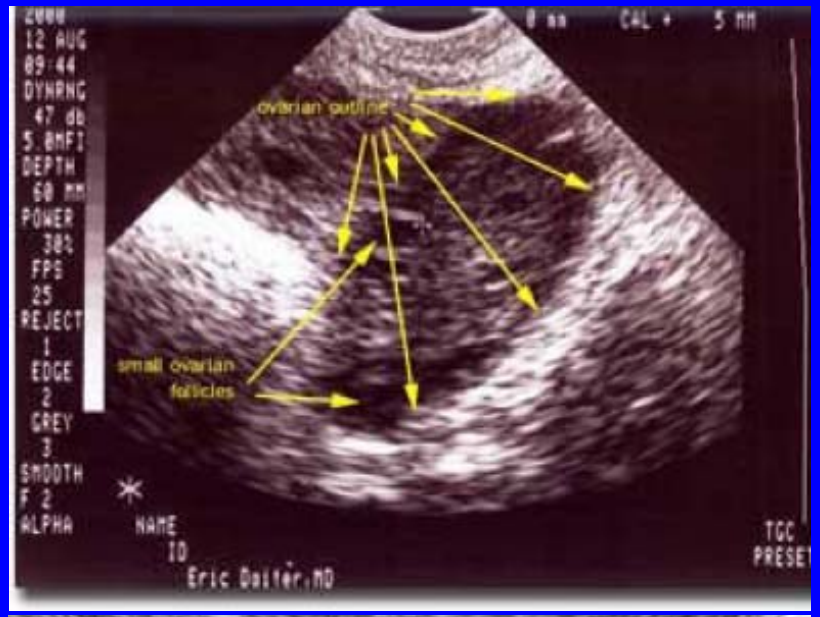


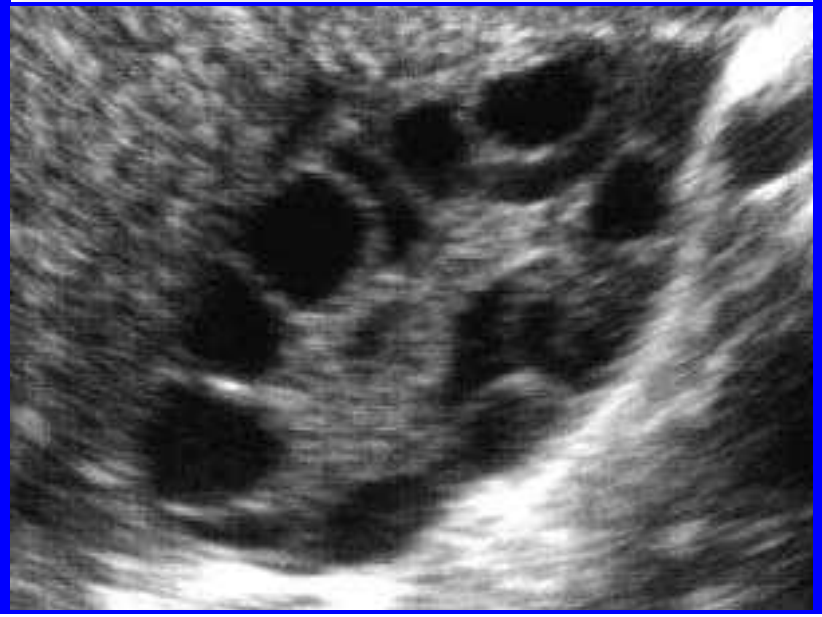
Screening of Ovarian Cancer

- Pelvic and rectal exam
- CA125 test
- Transvaginal sonography

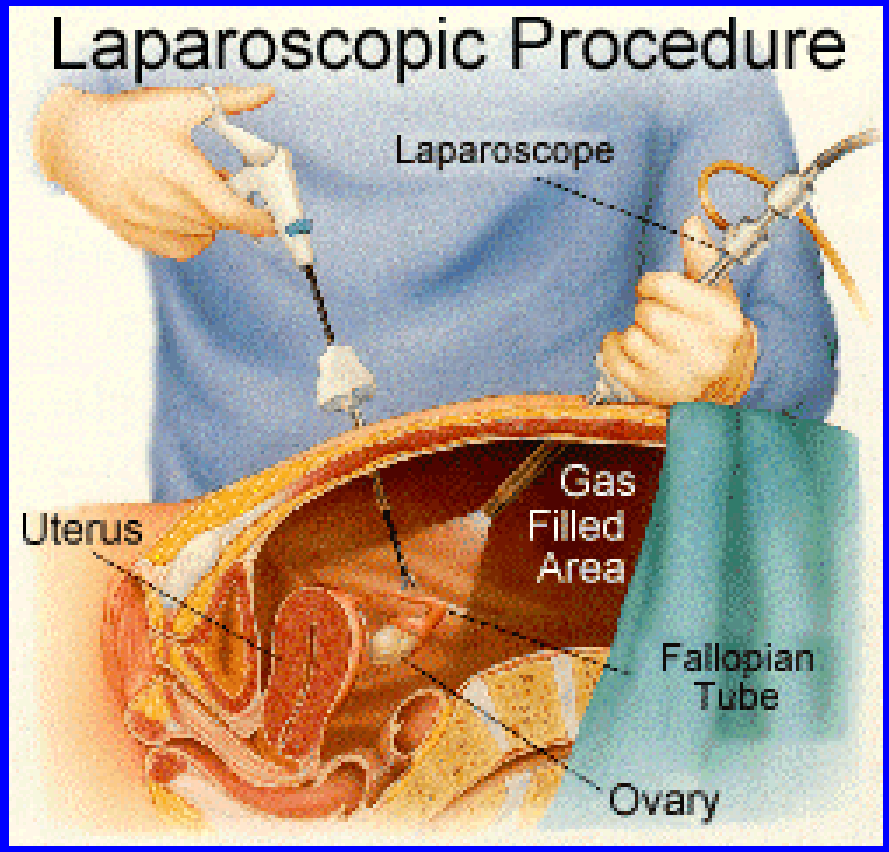
Transvaginal Sonography

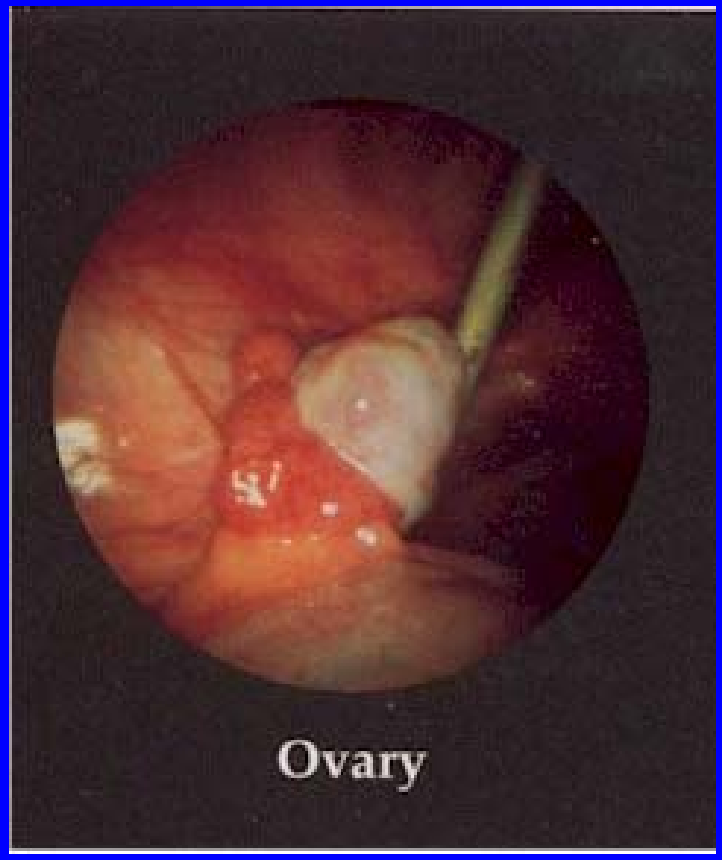






Diagnostic Laparoscopy





Complication Rate = 0.5 – 1%

Detection and Treatment

- Screening
  - Pelvic exam
  - CA125 test
  - Transvaginal ultrasound
- Diagnosis
  - Diagnostic laparoscopy
- Treatment:
  - Surgery, radiation therapy, chemotherapy
- 5 year survival
  - Localized disease: 93% (20% diagnosed at this stage)

Screening Scenarios

- Scenario #1:
  - Screen 1,000,000 women with CA125
    - p = .0001 (100 cancers)
    - Se=35%, Sp=98.5%
    - Cost = $30
  - Follow with laparoscopy
    - Complication rate = 1%
    - Cost=$2,000
- TP=35 FP=14,999 Complications=150
- PPV =0.23% NPV =99.99%
- Cost per cancer found = $1,716,200

Screening Scenarios

- Scenario #2:
  - Screen 1,000,000 women with transvaginal US
    - P = .0001 (100 cancers)
    - Se=100%, Sp=96%
    - Cost = $150
  - Follow with laparoscopy
    - Complication rate = 1%
    - Cost=$2,000
- TP=100 FP=39,996 Complications=401
- PPV =0.25% NPV =100%
- Cost per cancer found = $300,672

Screening Scenarios

- Scenario #3:
  - Screen 1,000,000 women >age 50 with TVUS
    - P = .0005 (500 cancers)
    - Se=100%, Sp=96%
    - Cost = $150
  - Follow with laparoscopy
    - Complication rate = 1%
    - Cost=$2,000
- TP=500 FP=39,980 Complications=405
- PPV =1.24% NPV =100%
- Cost per cancer found = $60,670

Screening Scenarios

- Scenario #3 cont.:
  - Screen 1,000,000 women > age 50 with TVUS
    - P = .0005 (500 cancers)
    - Se=100%, Sp=??%
    - Cost = $150
  - How high does Sp need to be for PPV to reach 25%?
    - Sp = 99.985%

Does Ultrasound Screening Work?

- Two studies of over 10,000 low-risk women:
  - The positive predictive value was only 2.6%
  - Ultrasound screening of 100,000 women over age 45 would:
    - Detect 40 cases of ovarian cancer,
    - Result in 5,398 false positives
    - Result in over 160 complications from diagnostic laparoscopy
  - Jacobs I. Screening for early ovarian cancer. Lancet; 2:171-172, 1988.

Ongoing Clinical Trials

- United Kingdom
  - 200,000 postmenopausal women
    - CA 125 level plus transvaginal ultrasound examination
    - Transvaginal ultrasound alone
    - No screening
- United States:
  - 37,000 women (aged 55–74)
    - Annual CA 125 level and transvaginal ultrasound examination
    - No screening
- Europe:
  - 120,000 postmenopausal women
    - No screening,
    - Transvaginal ultrasound at intervals of 18 months
    - Transvaginal ultrasound at intervals of 3 years

http://www.mja.com.au/public/issues/178_12_160603/and10666_fm.pdf

Challenge

Better screening methods to detect early stages of ovarian cancer

Cancer Screening Exams

- Cellular Changes
  - Pap smear
- Serum Proteins
  - PSA
  - CA125
  - OvaCheck
- Genetic Changes
  - HPV DNA

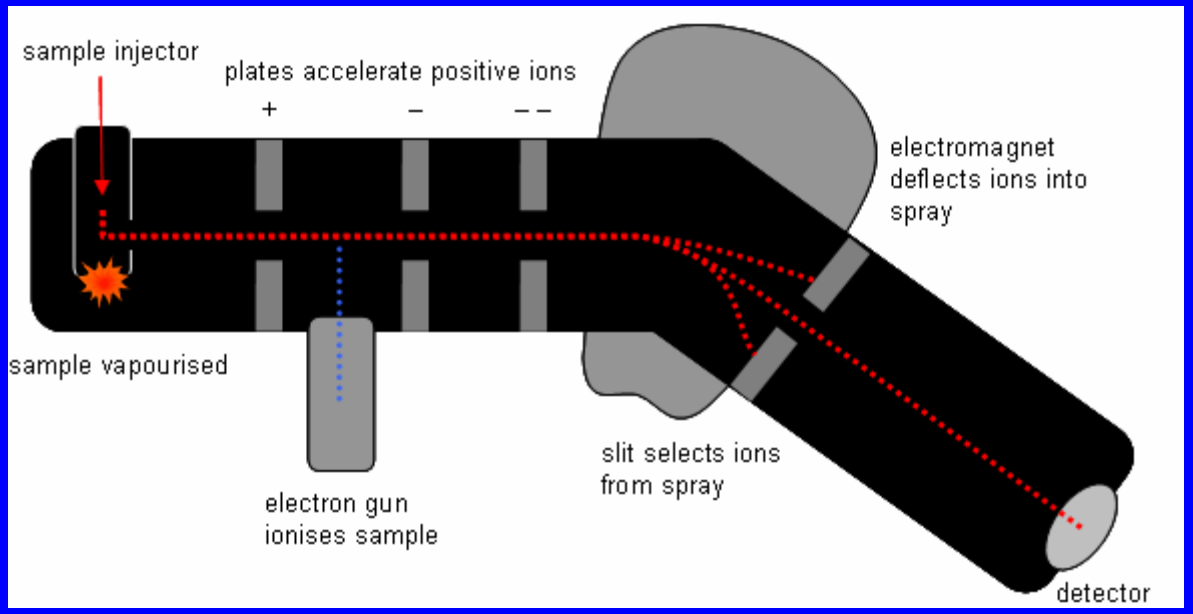
New Screening Tool

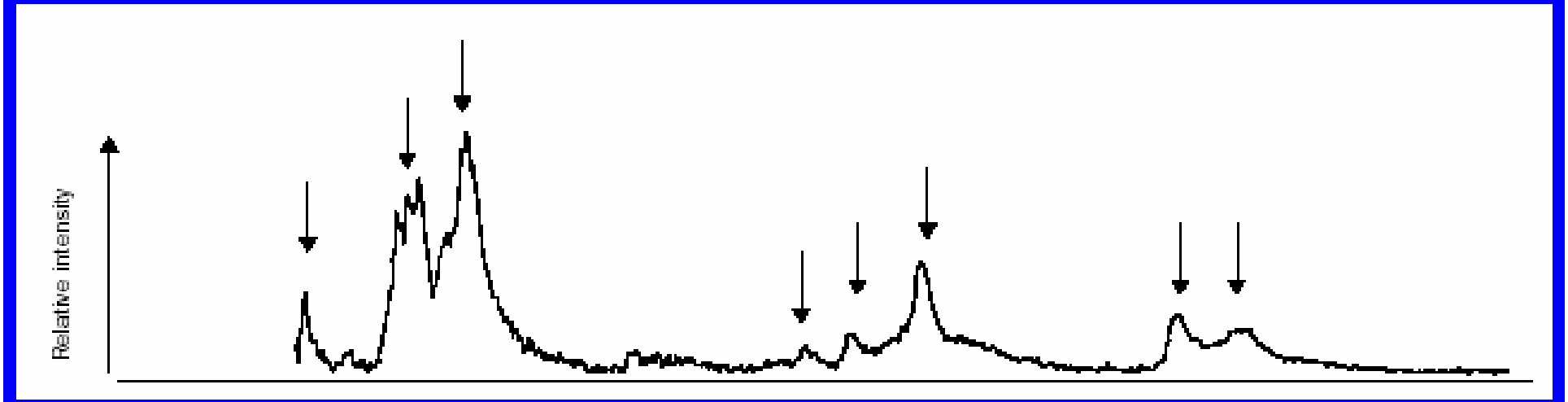
- Current screening tests look for 1 protein:
  - CA125
  - PSA
- Many serum proteins
- Can complex fingerprint predictive of cancer can be identified?
- PROTEOMICS:
  - Don’t try to understand disease mechanisms
  - Use proteomics to analyze patterns made by all proteins in the blood, without even knowing what they are

How do we measure serum proteins?

- Mass Spectrometry:
  - Serum proteins are vaporized, given an electric charge and propelled down a tube
  - How fast they make the trip depends on their mass
  - Produces graph that shows distribution of masses in the sample
  - Use computer program to analyze patterns and distinguish blood from patients with cancer and from those without

Proteomics: Mass Spectrometer





Mechanisms of disease

Use of proteomic patterns in serum to identify ovarian cancer

Emanuel F Petricoin III, Ali M Ardekani, Ben A Hitt, Peter J Levine, Vincent A Fusaro, Seth M Steinberg, Gordon B Mills, Charles Simone, David A Fishman, Elise C Kohn, Lance A Liotta

Summary

Background New technologies for the detection of early-stage ovarian cancer are urgently needed. Pathological changes within an organ might be reflected in proteomic patterns in serum. We developed a bioinformatics tool and used it to identify proteomic patterns in serum that distinguish neoplastic from non-neoplastic disease within the ovary.

Methods Proteomic spectra were generated by mass spectroscopy (surface-enhanced laser desorption and ionisation). A preliminary "training" set of spectra derived from analysis of serum from 50 unaffected women and 50 patients with ovarian cancer were analysed by an iterative searching algorithm that identified a proteomic pattern that completely discriminated cancer from non-cancer. The discovered pattern was then used to classify an independent set of 116 masked serum samples: 50 from women with ovarian cancer, and 66 from unaffected women or those with non-malignant disorders.

Findings The algorithm identified a cluster pattern that, in the training set, completely segregated cancer from non-cancer. The discriminatory pattern correctly identified all 50 ovarian cancer cases in the masked set, including all 18 stage I cases. Of the 66 cases of non-malignant disease, 63 were recognised as not cancer. This result yielded a sensitivity of 100% (95% CI 93–100), specificity of 95% (87–99), and positive predictive value of 94% (84–99).

Interpretation These findings justify a prospective population-based assessment of proteomic pattern technology as a screening tool for all stages of ovarian cancer in high-risk and general populations.

Lancet 2002; **359:** 572–77

Introduction

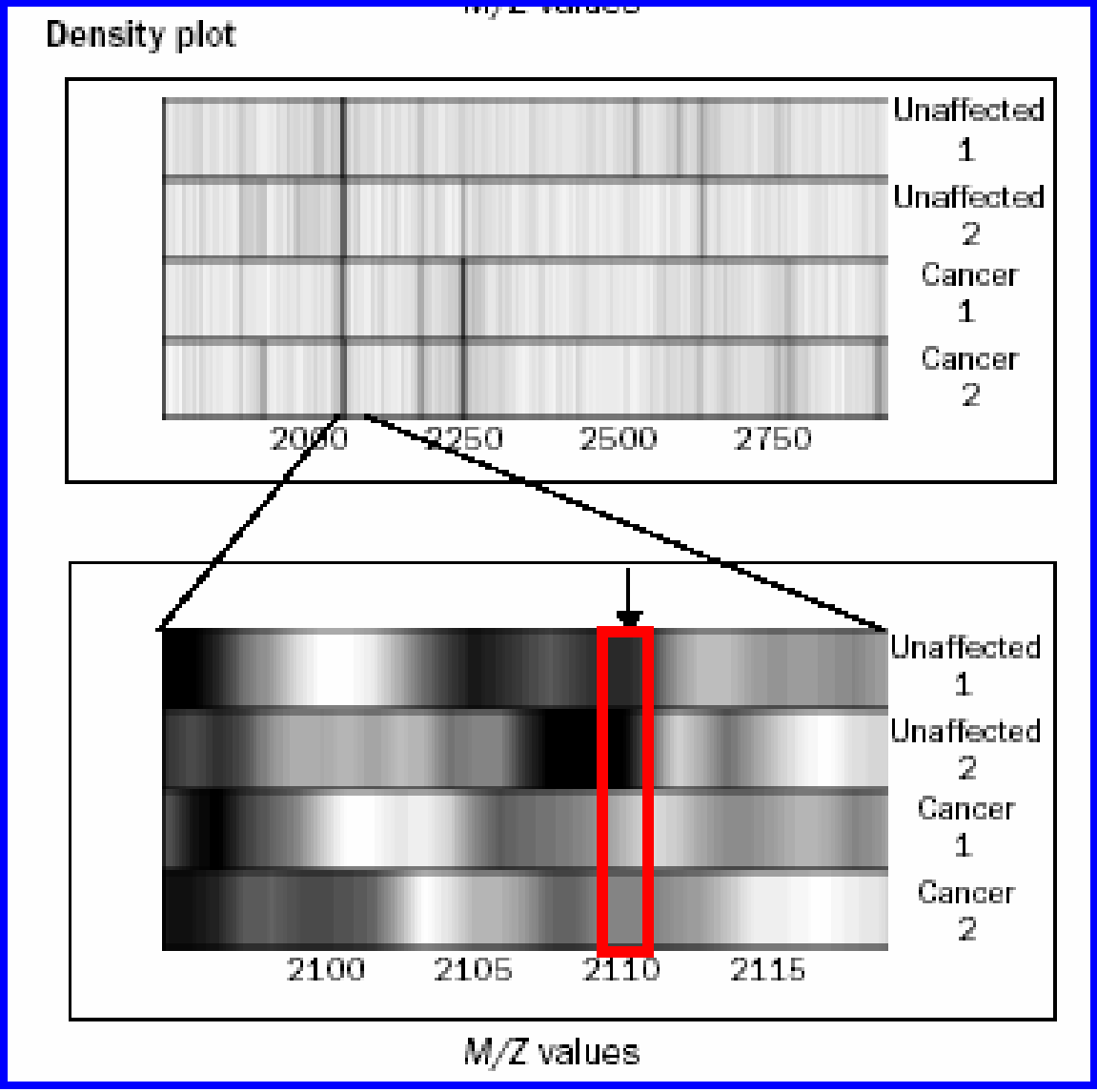
Application of new technologies for detection of ovarian cancer could have an important effect on public health,[1] but to achieve this goal, specific and sensitive molecular markers are essential.[1–3] This need is especially urgent in women who have a high risk of ovarian cancer due to family or personal history of cancer, and for women with a genetic predisposition to cancer due to abnormalities in predisposition genes such as *BRCA1* and *BRCA2*. There are no effective screening options for this population.

Ovarian cancer presents at a late clinical stage in more than 80% of patients,[1] and is associated with a 5-year survival of 35% in this population. By contrast, the 5-year survival for patients with stage I ovarian cancer exceeds 90%, and most patients are cured of their disease by surgery alone.[1–6] Therefore, increasing the number of women diagnosed with stage I disease should have a direct effect on the mortality and economics of this cancer without the need to change surgical or chemotherapeutic approaches.

Cancer antigen 125 (CA125) is the most widely used biomarker for ovarian cancer.[1–6] Although concentrations of CA125 are abnormal in about 80% of patients with advanced stage disease, they are increased in only 50–60% of patients with stage I ovarian cancer.[1–6] CA125 has a positive predictive value of less than 10% as a single marker, but the addition of ultrasound screening to CA125 measurement has improved the positive predictive value to about 20%.[6]

Low-molecular-weight serum protein profiling might reflect the pathological state of organs and aid in the early detection of cancer. Matrix-assisted laser desorption and ionisation time-of-flight (MALDI-TOF) and surface-enhanced laser desorption and ionisation time-of-flight (SELDI-TOF) mass spectroscopy can profile proteins in this range.[6–9] These profiles can contain

Comparative Analysis



Useful M/Z:

534

989

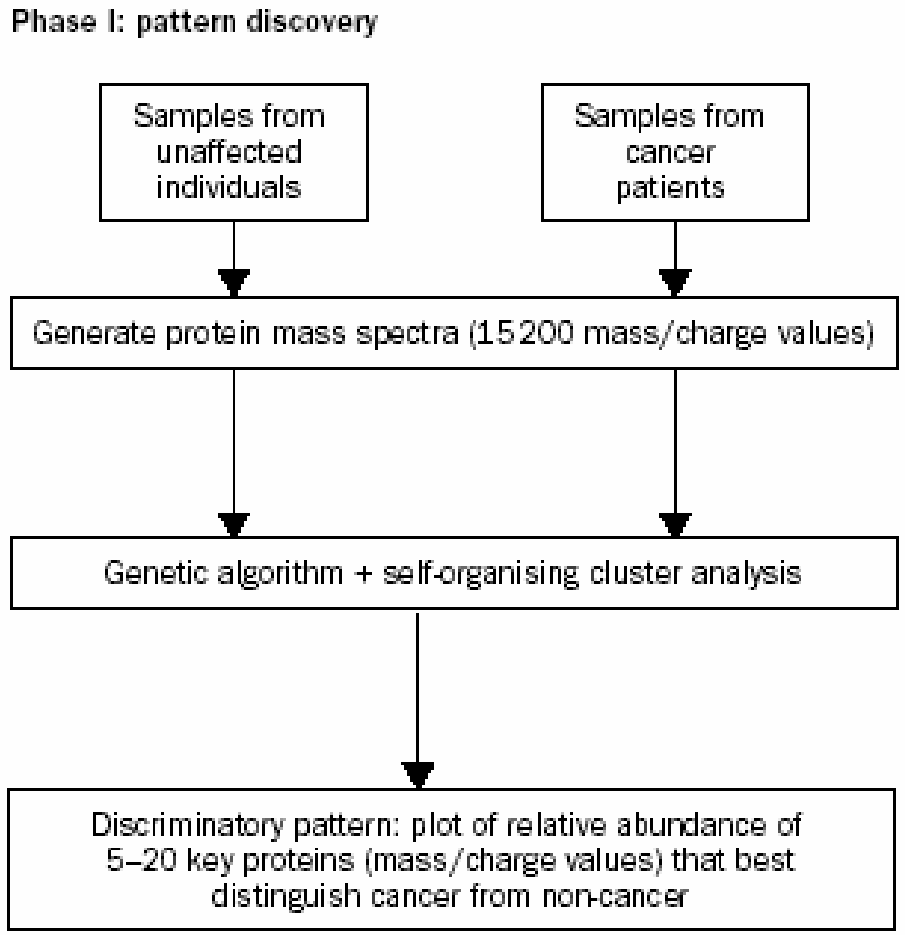
2111

2251

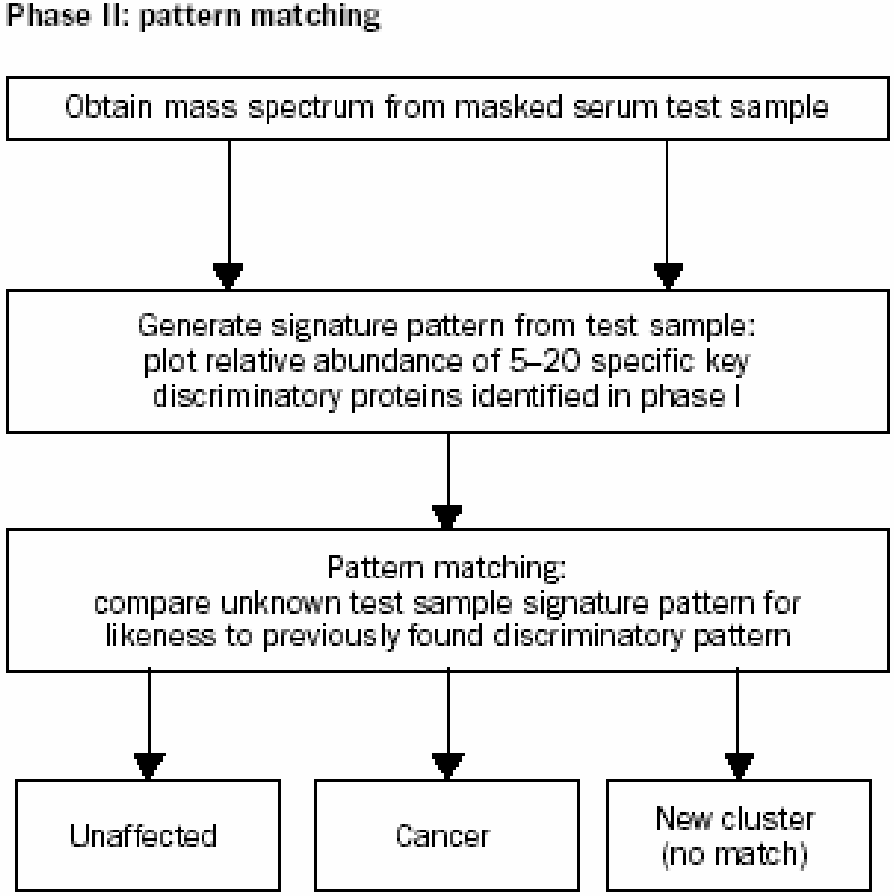
2465

Data Analysis

Training



Validation



OvaCheck

- Quest Diagnostics and LabCorp:
  - Will analyze blood samples sent by doctors, rather than sell test kits to doctors and hospitals
  - Tests performed at a central location do not require F.D.A. approval
  - Will be available in a few months
  - Cost: $100-$200

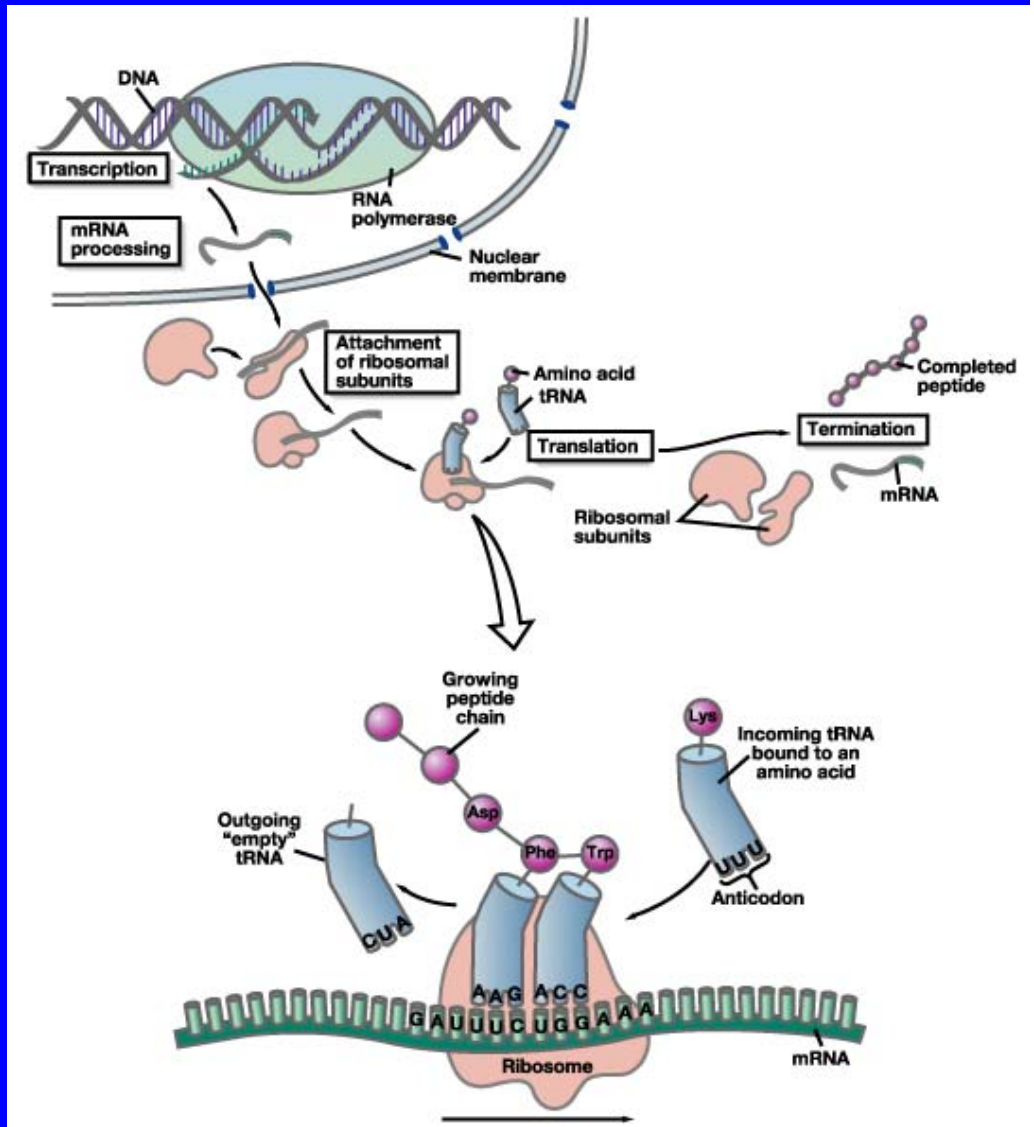
Response

- Dr. Eleftherios P. Diamandis, head of clinical biochem at Mount Sinai Hospital in Toronto.
  - "If you don't know what you're measuring, it's a dangerous black-box technology... They are rushing into something and it could be a disaster.“
- Dr. Nicole Urban, head of gynecologic cancer research at the Fred Hutchinson Cancer Research Center in Seattle.
  - "Certainly there's no published work that would make me tell a woman she should get this test.“
- Dr. Beth Karlan, director of gynecologic oncology at Cedars-Sinai Medical Center
  - "Before you mass-market to the uninformed, fearful population, it should be peer-reviewed,"
  - When asked whether she would recommend her patients not get tested, she said: "It doesn't matter what I recommend. They are going to do it anyway."

http://www.ovarian.org/press.asp?releaseID=263

Gene Expression Analysis

- Human Genome
  - 30,000 unique genes
  - Which genes are active?
- DNA Microarrays
  - Tool to study gene expression
  - Which genes are turned on or off as cells grow, divide, respond to hormones, etc



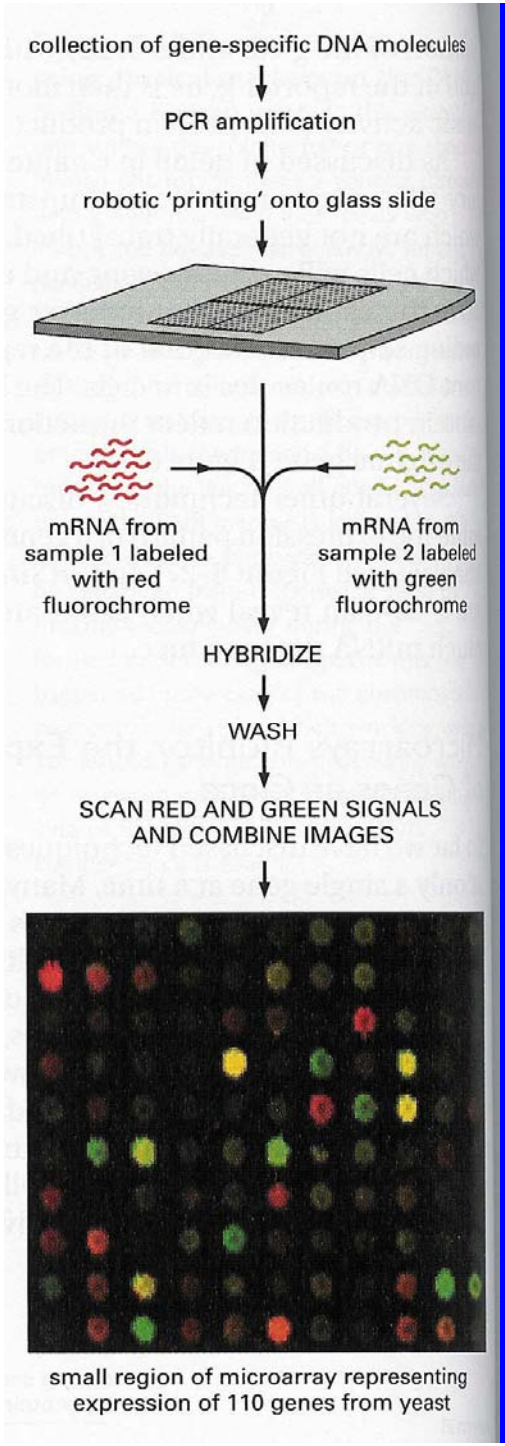
What is a DNA Microarray?

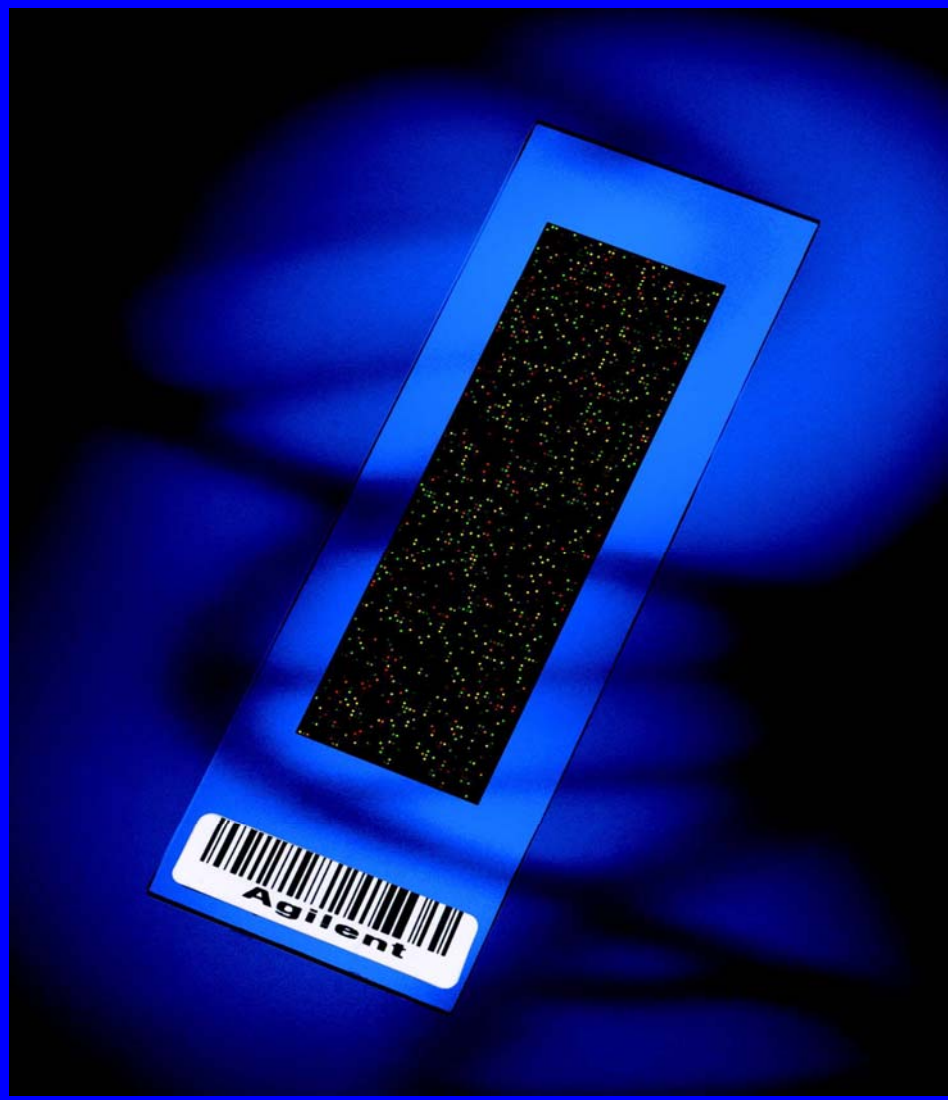
- Glass slide
  - Large number of DNA fragments
  - Each contains nucleotide sequence to probe for a specific gene
    - Short oligos synthesized on surface of glass wafer
    - Large DNA fragments generated by PCR and spotted onto slide by robot
  - Each gene has unique physical address on slide

How Do We Use a DNA Microarray?

- Extract mRNA from cells under study
- Convert mRNA to cDNA
- Label cDNA with fluorescent probe
- Incubate labeled cDNA with microarray
- Wash slide to remove unbound cDNA
- Scan slide with laser scanning fluorescence microscope
- Determine which genes are expressed in test sample

DNA Microarrays





From: Molecular Biology of the Cell

New screening technologies

- New screening technologies
  - Proteomics
  - DNA microarrays