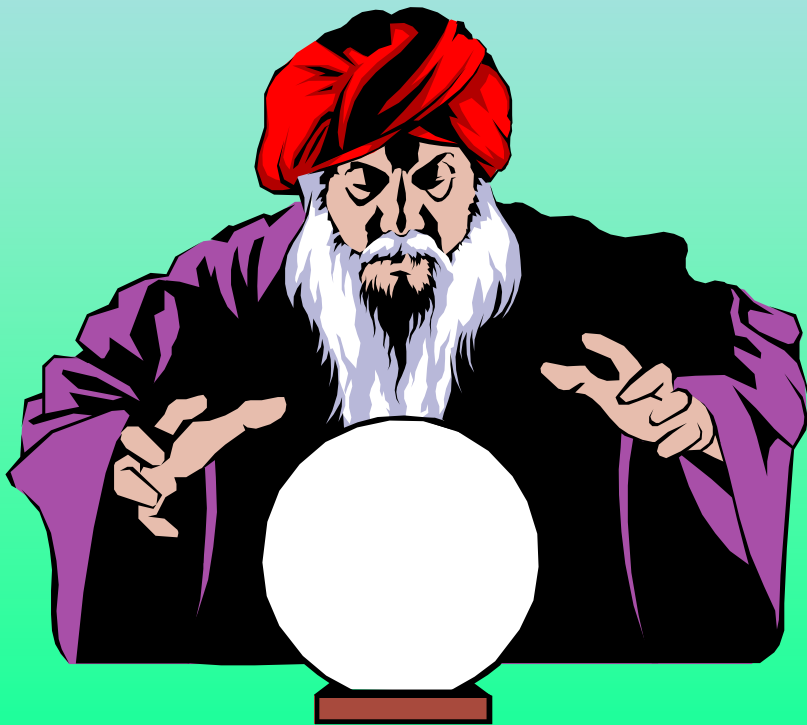


HAEMOSTASIS TECHNIQUES OF TOMORROW



Thomas Exner
Haematech Research
Hornsby, NSW

PLAN

- BRIEF HISTORY
 - Technical milestones
 - Tests
 - Instruments
- Drivers of change
- Routine vs research
- Latest innovations

MILESTONES IN THE DEVELOPMENT OF HAEMOSTASIS TECHNIQUES

1878: Vierordt - Capillary tube blood clot tests.

1889: Hayem - Test tube clotting times (3-20').

1914: Howell - Oxalate anticoagulant.

1935: Ivy - Skin bleeding time

1939: Nygaard - Photoelectric coagulometer>>>

1948: Hartert - Thrombelastography>>>

1952: Astrup - Fibrin agarose plates>>>

1962: Born - Light transmission aggregometry>>>

1970: Blomback-Chromogenic substrates

SEDIMENTING BEAD METHOD

- Sephadex beads suspended in dilute fibrinogen solution in test tubes settle out at rates depending on viscosity, their particle size, density, etc.
- Turbidity of such a suspension is reduced with time as the beads settle out.
- Thrombin can clot the fibrinogen and stop bead sedimentation. Thus final turbidity (due to the beads stuck in the clot) is inversely proportional to clotting time and proportional to thrombin concentration.
- Exner T, Koppel JL. Fibrinogen-fibrin conversion as determined by polymer bead sedimentation technique. *Experientia*.1972;28;1421.

CLOTTING TESTS:

Whole blood clotting time

Plasma recalcification time

1935: Quick-prothrombin time test

1961: Proctor & Rapaport-APTT

Factor assays.

Thrombin/thromboplastin generation tests

1970: *Blomback-Chromogenic substrates*

Tricky factor assays

Assays for FXa, IIa, ATIII, heparin etc

Latex agglutination tests (FDP's)

1995: *Hemker-Thrombography*

INSTRUMENTS

Fibrometer (BBL)

Electra (MLA)

Coagamate (Gen Diagnostics)

STAR series (Stago)

Automated Coagulation Lab* (IL)

Thrombelastogram (TEG, ROTEM, etc)

Point of care devices

*Thrombinoscope (Synapse)

*Chromo or Fluoro substrate enabled

OTHER TECHNIQUES

Immuno/agarose gel methods

Immunodiffusion

Laurells

ELISA/Bioassays

Surface plasmon resonance

Flow cytometry (incl. Luminex)

Lab on a “chip”

Proteomics (MALDI/TOF)

Microarrays (Protein, cDNA)

MICROCOAGULATION INSTRUMENTS-“Lab on a Chip”

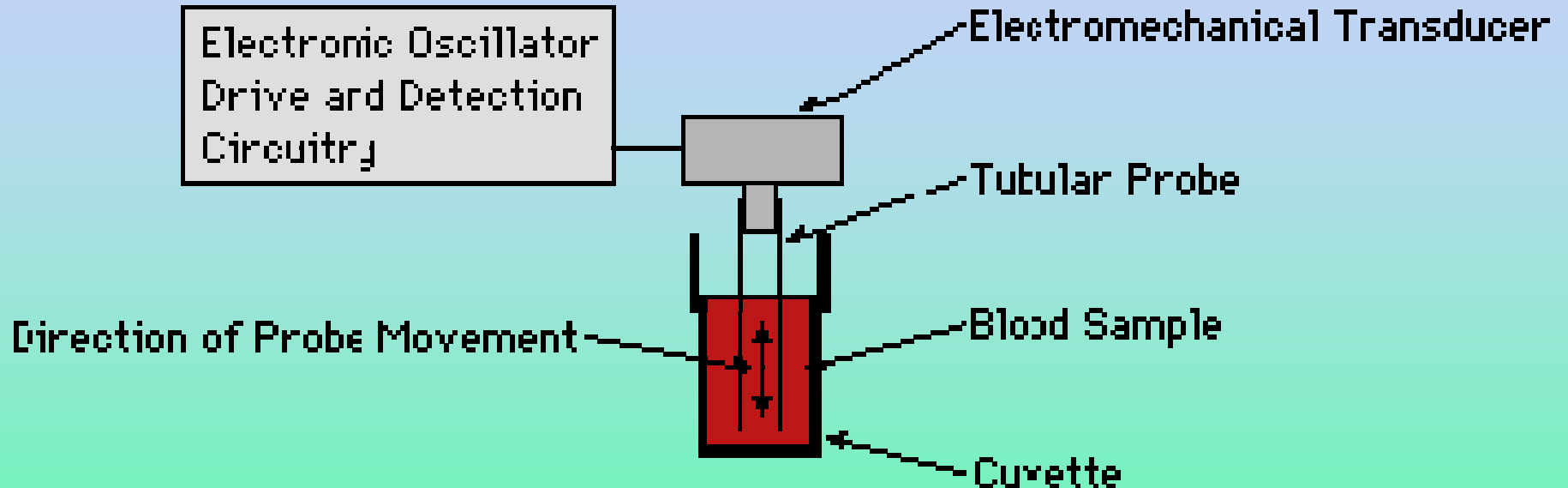
- Thrombolysis Analyzer System (TAS): Magnetic beads/laser scanner.
 - Protine (ITC): Multiple capillary flow/LED scanner.
 - Coumatrak: Capillary flow/laser scanner. (BM>Roche)
 - CoaguChek I, II, XS (Roche): Chromogenic>
>electrochemical sensing.
-
- Mainly developed for home monitoring of INRs.
 - Now also used for hirudin testing using ecarin.
 - APTT tests available but poor correlation with plasma methods.
 - Not yet suitable for other tests.

WHOLE BLOOD CLOT SENSING DEVICES

- MEDTRONIC
- SONOCLOT
- TEG (HAEMOSCOPE)
- ROTEM (PENTAPHARM)
- FREE OSCILLATION RHEOMETRY(MEDIROX)

- Mainly used for surgical theatres and critical care for assessment of fibrinogen, platelet, overall clotting, fibrinolytic and heparin status.

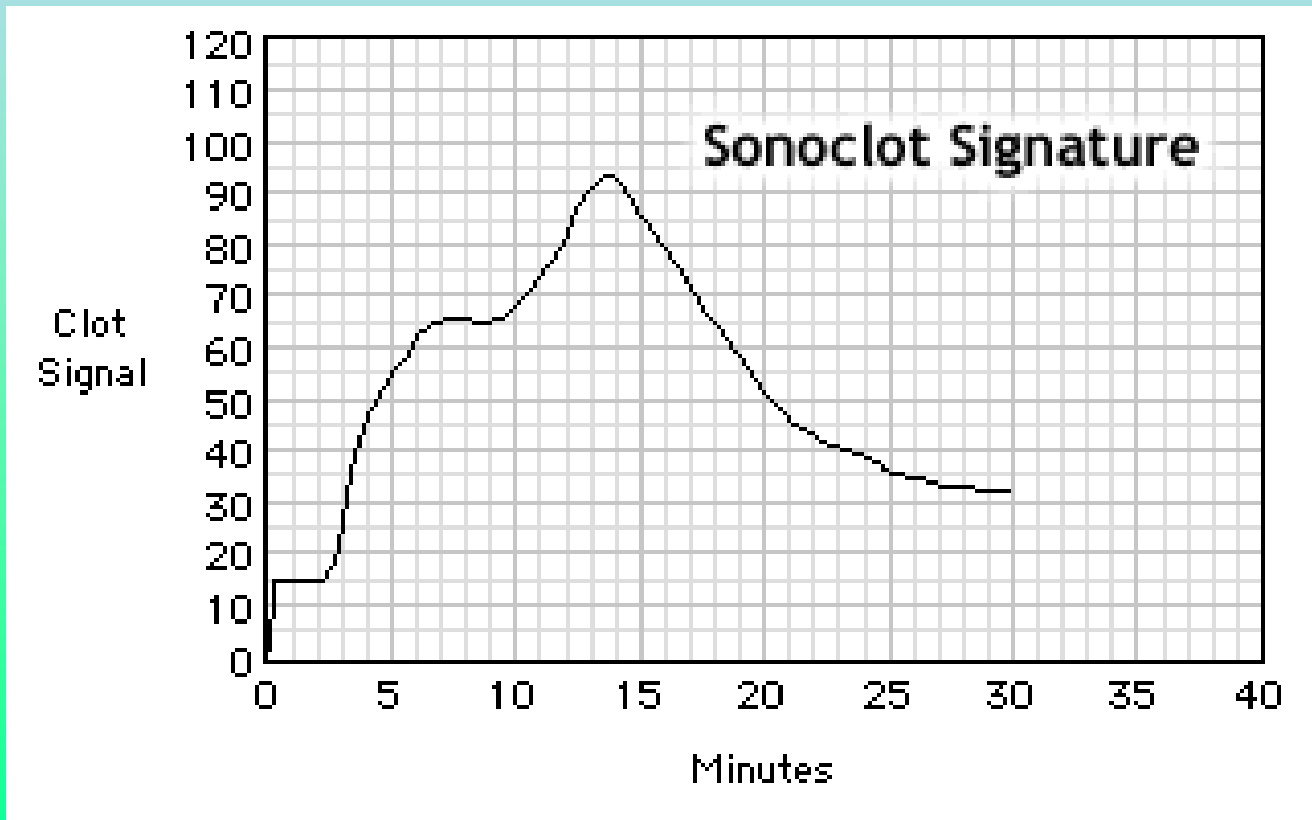
SONOCLOT ANALYZER



The detection mechanism within the Sonoclot Analyzer responds to mechanical changes that occur within the blood sample. This mechanism consists of a tubular probe that oscillates up and down within a blood sample. The electronic drive and detection circuitry senses the resistance to motion that the probe encounters from the blood sample and generates an analog electronic signal.

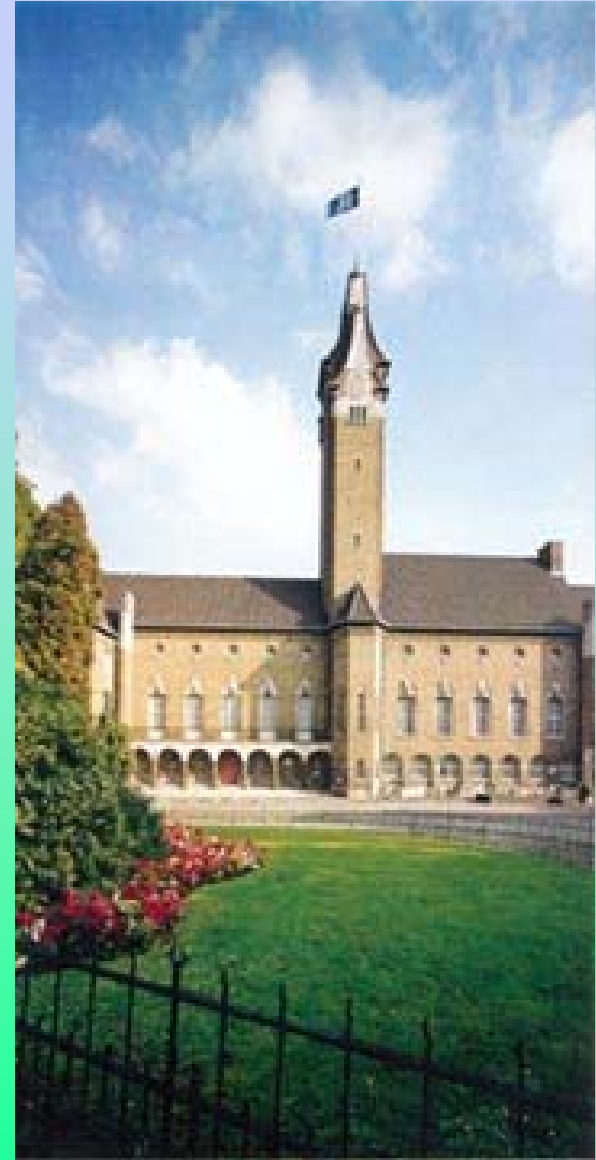
From Sienco website:

“The Sonoclot whole blood analysis is unlike other coagulation tests which either suffer from the lack of cellular elements or only provide data on separate components of the blood, thereby overlooking important interactions essential to the clinical evaluation of hemostasis. The Sonoclot Analyzer rapidly provides accurate information on the entire hemostasis process including coagulation, fibrin gel formation, clot retraction (platelet function) and fibrinolysis”.





H.C. HEMKER, Maastricht, NL



PRINCIPLE OF THROMBOGRAPHY

Citrated test plasma (with 150×10^9 pL/L)

+ Calcium + Tissue factor (0.5pM)

(+/- Activated protein C or TM)

>>> Thrombin

+ Fluorogenic substrate(Z-Gly-Gly-Arg-AMC) >>> AMC



Test carried out in thermostatted microwells,
monitored by Fluoroskan Ascent (fluoro microplate reader).
Results analysed by “Thrombinoscope” computer software.

Total amount of thrombin formed = area under curve
= Endogenous Thrombin Potential (ETP)

Endogenous Thrombin Potential

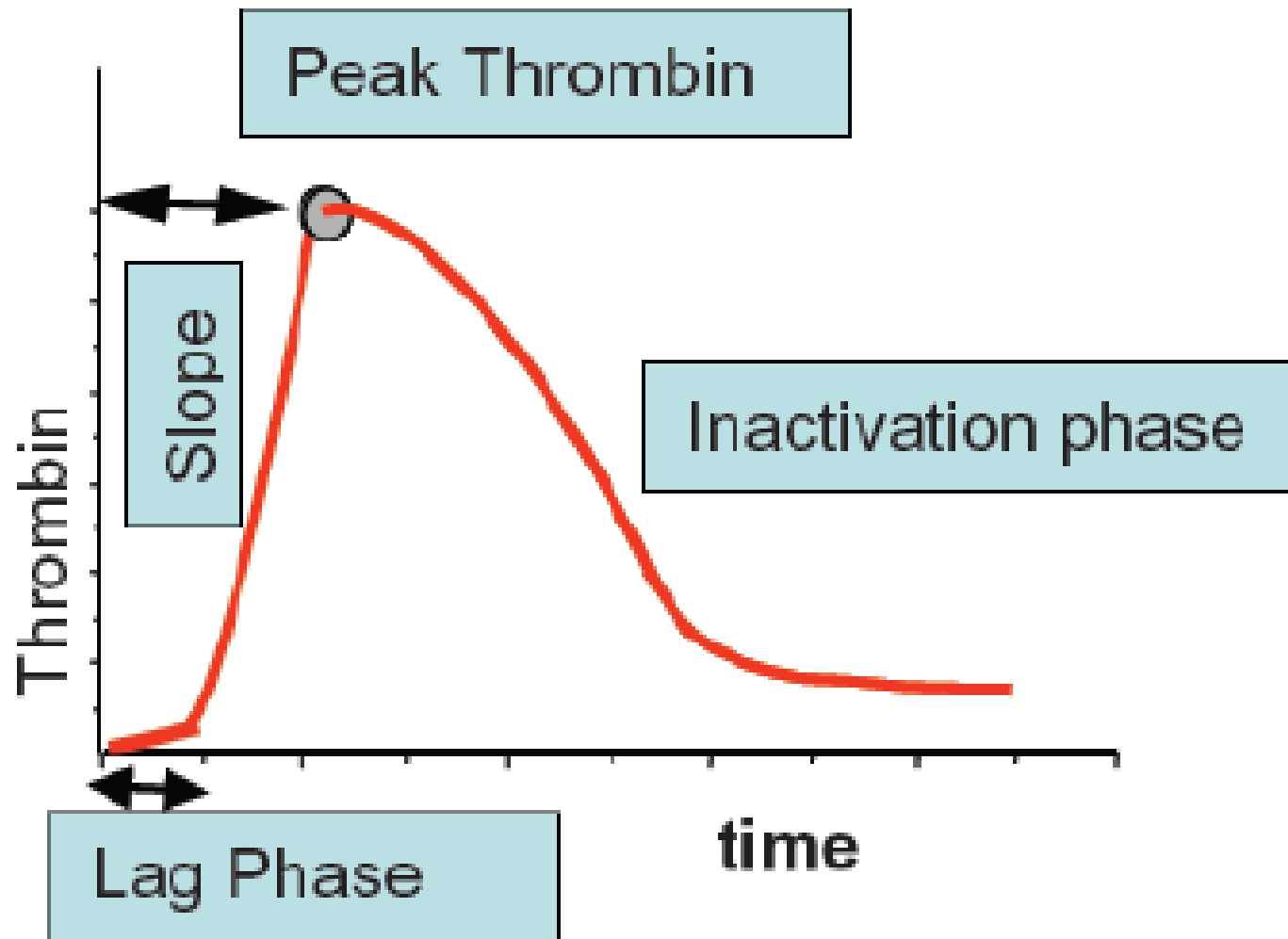
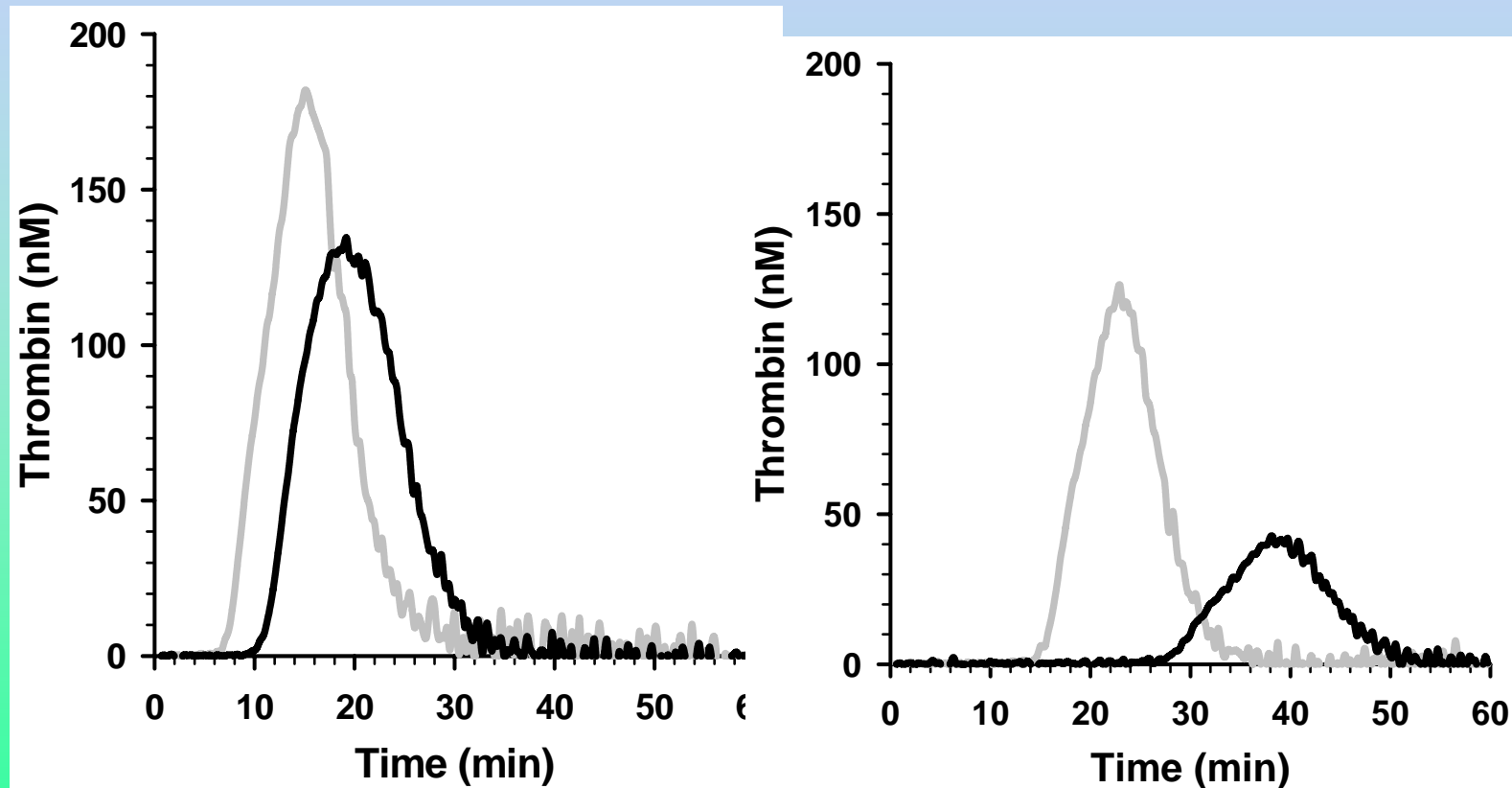
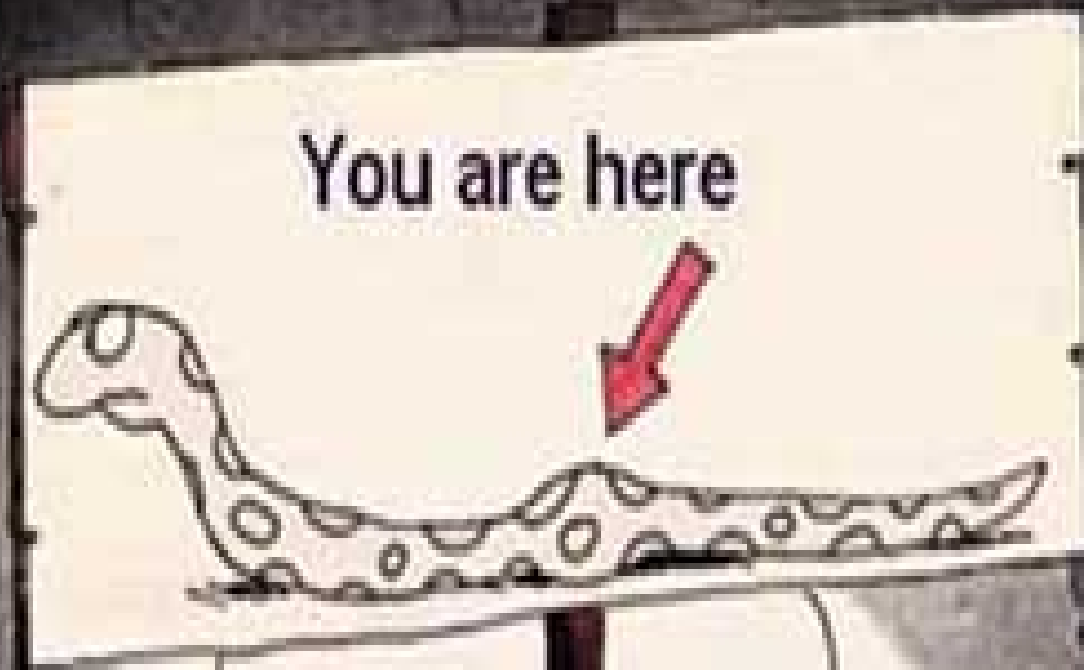


Fig. 1: *Effect of blood collection on thrombin activity.* Thrombograms observed with PRP prepared 30 min after blood collection into Vacutainer (gray lines) or Monovette (black lines) tubes in the absence (left) or presence (right) of 25 nM APC.



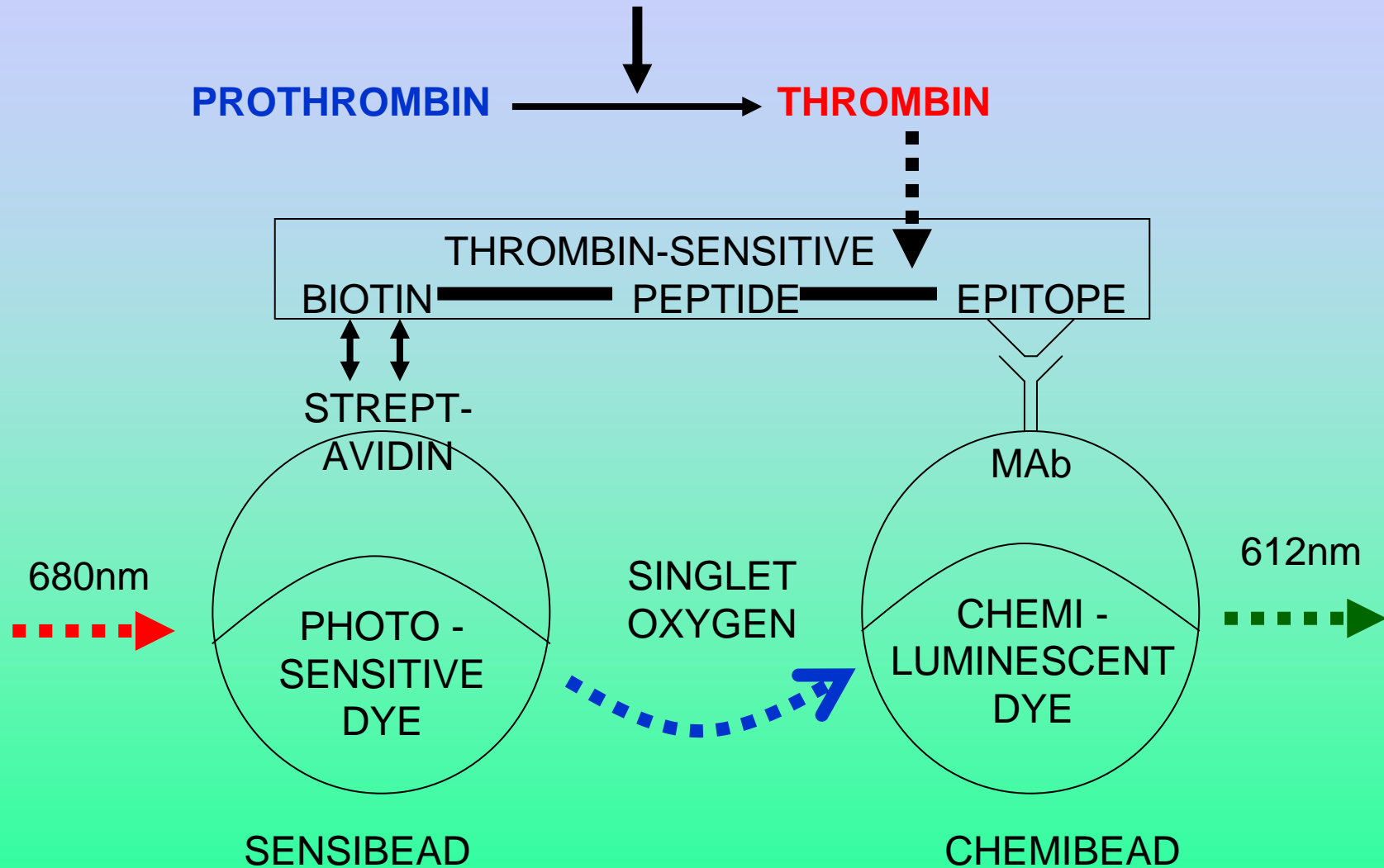
THROMBIN GENERATION TESTS

- The amount of thrombin formed may be more important to the development of a thrombus than the rate at which it is formed.(Why worry about fibrinogen levels ?).
- Standardisation of thrombin generation analysers is being actively pursued by ISTH subcommittees.
- New TGA instrument from Technoclone.
- (Wagner L, et al “First experiences with the measurement of thrombin generation on the Ceveron alpha in the routine lab”. ISTH meeting.PP-MO-551).



LUMINESCENT OXYGEN CHANNELING IMMUNOASSAY

LOCI PRINCIPLE AS ADAPTED FOR COAGULATION TESTS(SIEMENS HEALTHCARE)



FUNCTIONAL BLOOD COAGULATION ASSAYS BASED ON THE “LOCI” TECHNOLOGY

ISTH.2009, PP-MO-107: A.KAPPEL & G.CHRIST, SIEMENS, MARBURG, GERMANY

- LOCI runs on the “Dimension Vista” platform which is normally used for homogeneous immunoassays. Chemiluminescence response is linear over 4 log scale of concentration.
- For clotting tests reduction in signal is proportional to the amount of thrombin formed (obviously other enzymes can also be detected).
- Routinely uses 2-10ul sample volumes.
- In this particular paper the method was used to assay factor deficiencies in both intrinsic and extrinsic pathways.
- Also used for detecting direct thrombin inhibitors (Ecarin reagent)
- LOCI technology has potential for extension to functional coagulation assays.

SIEMENS

Dimension Vista® 1500 Intelligent Lab System



COMMENT FROM MARK TRISCOTT- VP-Reagent Development, Instrumentation Laboratories, Orangeburg, NY, USA

- Big coagulation instruments will get bigger.
- Small instruments will get smaller.
- Chemiluminescent coag analyser under development.
- Accustar immunoassay instrument using magnetic bead technology.
- Cepheid DNA for real time PCR to do II and V genotyping, from 50 ul of whole blood in 30 mins. They use a primer/probe technology called scorpions.
- Dedicated consumables and reagents, often in “cartridge” form.

TRENDS IN COAGULATION TESTING

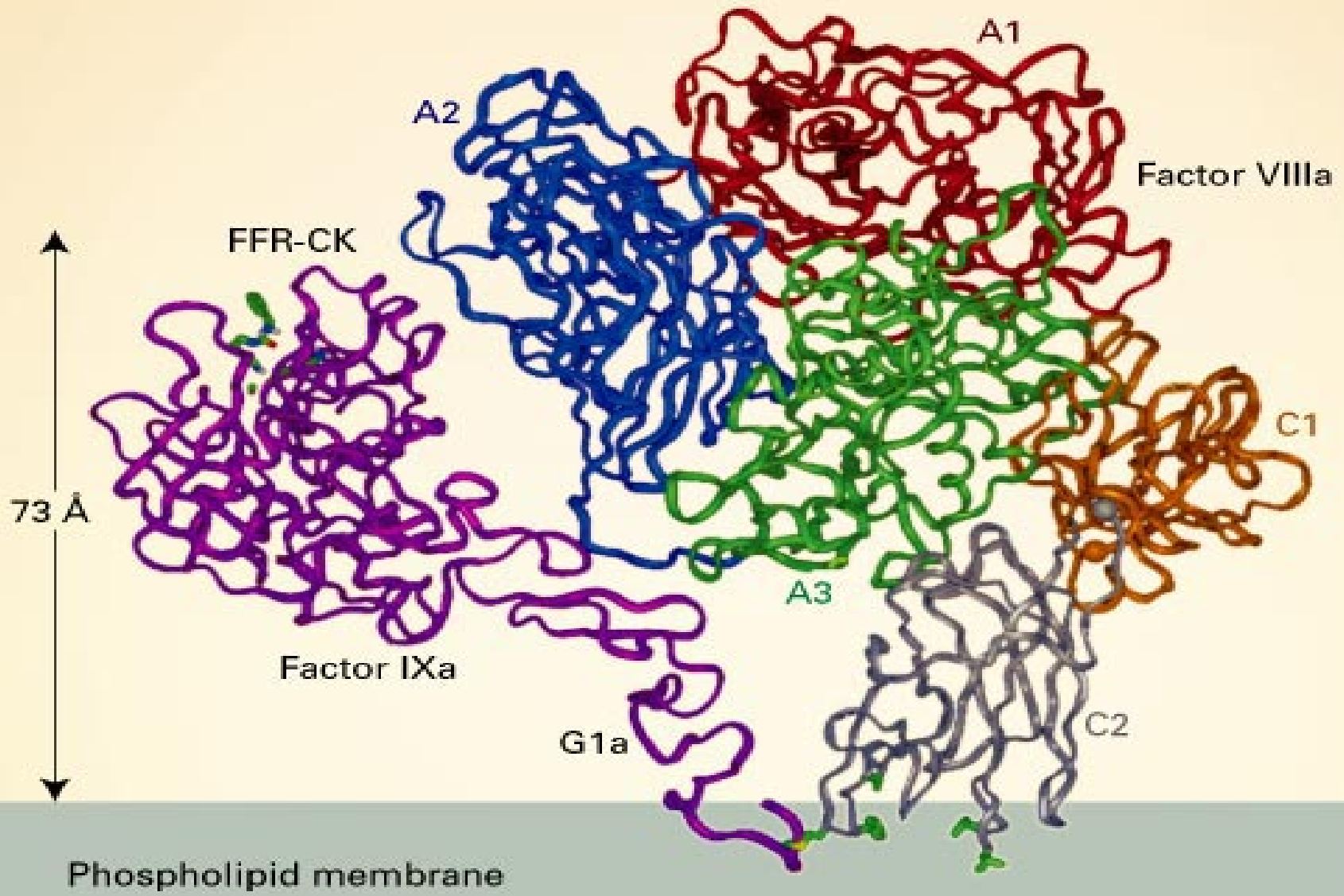
ROUTINE TESTS:

- Increased automation gives better quality results but increases reliance on suppliers
- Dedicated reagents in cartridges or in dried form reduce variation.
- Less demand for technical expertise
- Fewer opportunities for innovation.

- RESEARCH WORK:

- Major developments in analytical methods
- Molecular biology, information systems etc.

Complex of VIIIa and IXa



HOW USEFUL ARE COAGULATION TESTS ANYWAY?

- BLEEDING DISORDERS

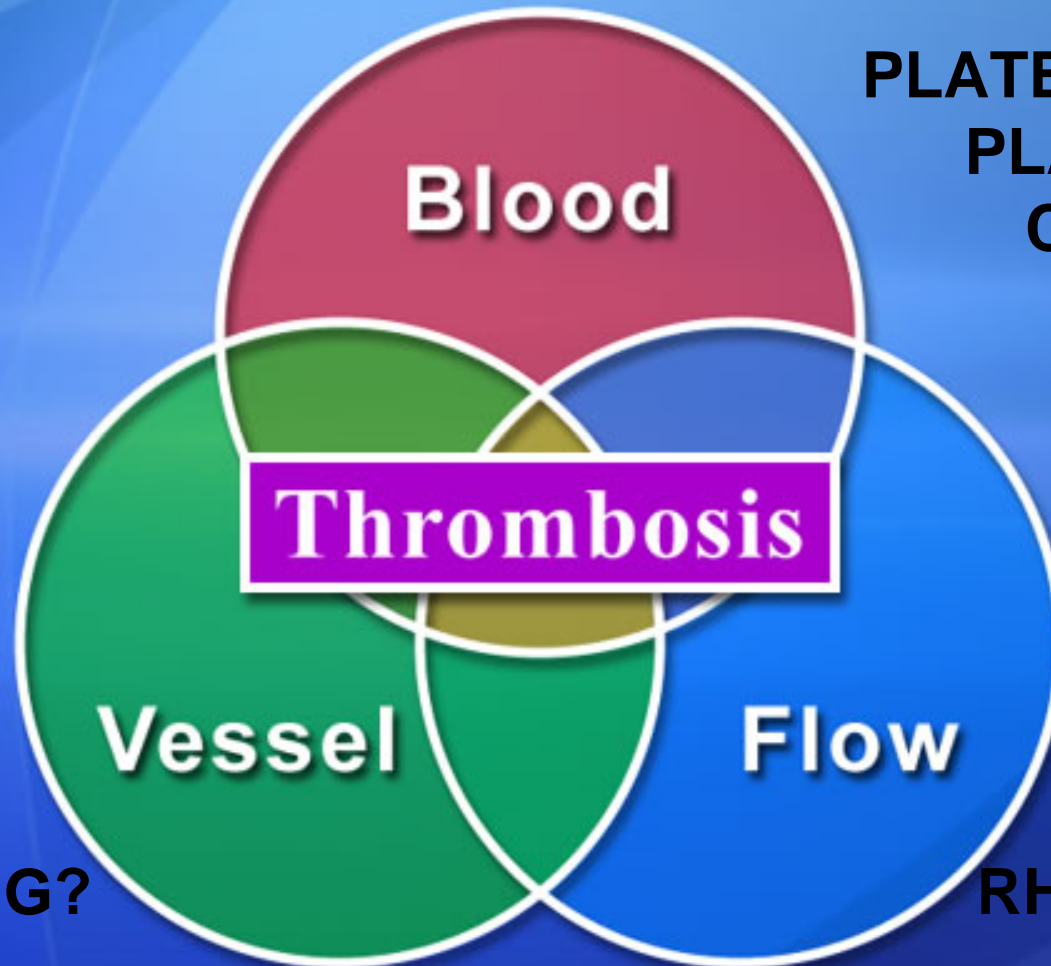
- Many patients have bleeding problems without known abnormalities.
- Most new anticoagulants (also TPA) do not require monitoring.
- Lupus paradox: Prolonged clotting tests due to lupus inhibitor do not cause bleeding.

- THROMBOTIC DISORDERS

- Factor V (Leiden) individuals do not show shortened life span. (Bertina)
- Thrombotic risk factors do not influence rate of DVT after hip and knee surgery (Joseph)
- Testing for inherited thrombophilia does not reduce the recurrence of venous thrombosis (Rosendaal).

WHAT ARE WE MISSING?

Virchow's triad



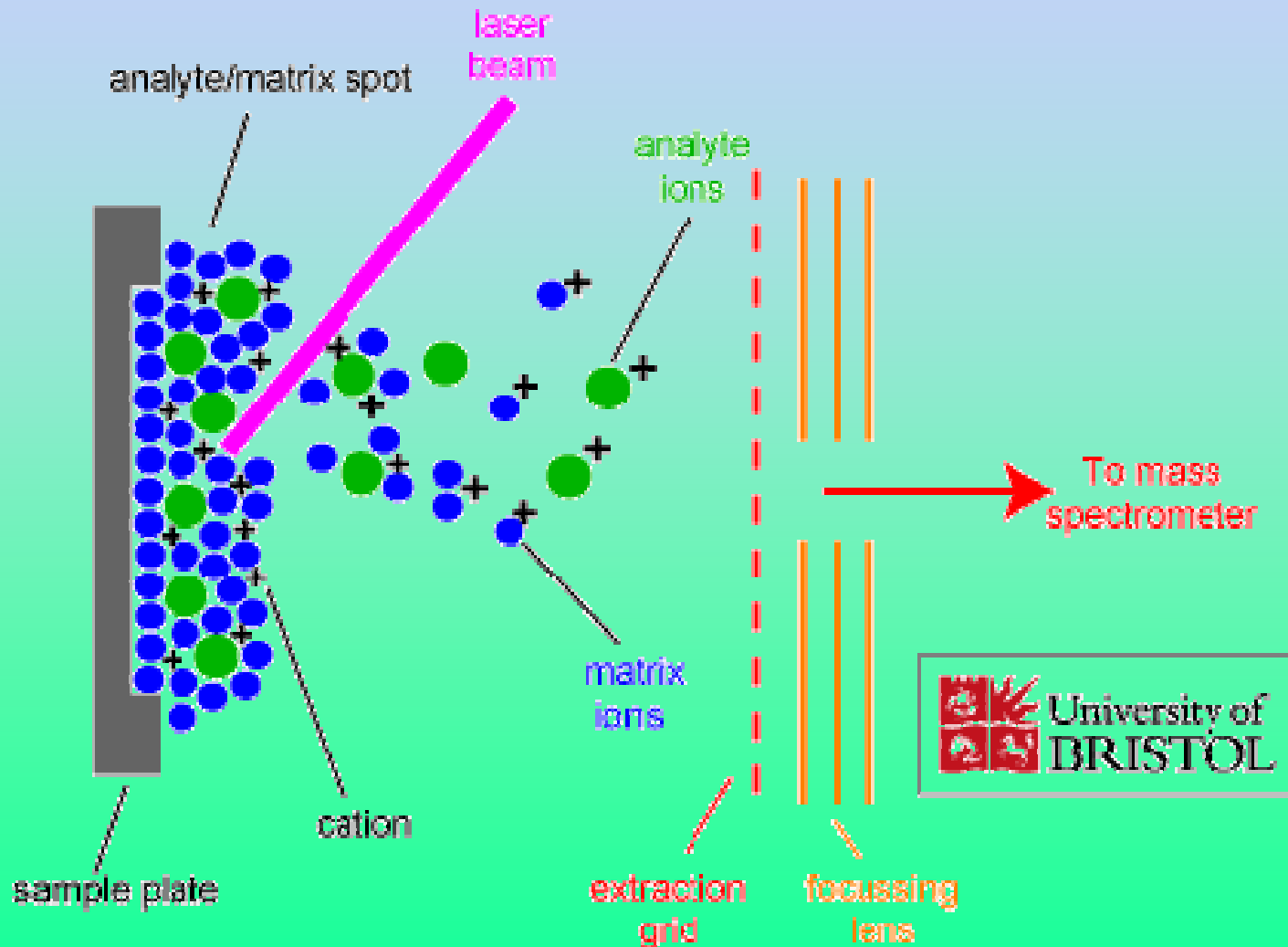
PLATELETS
PLASMA
CELLS

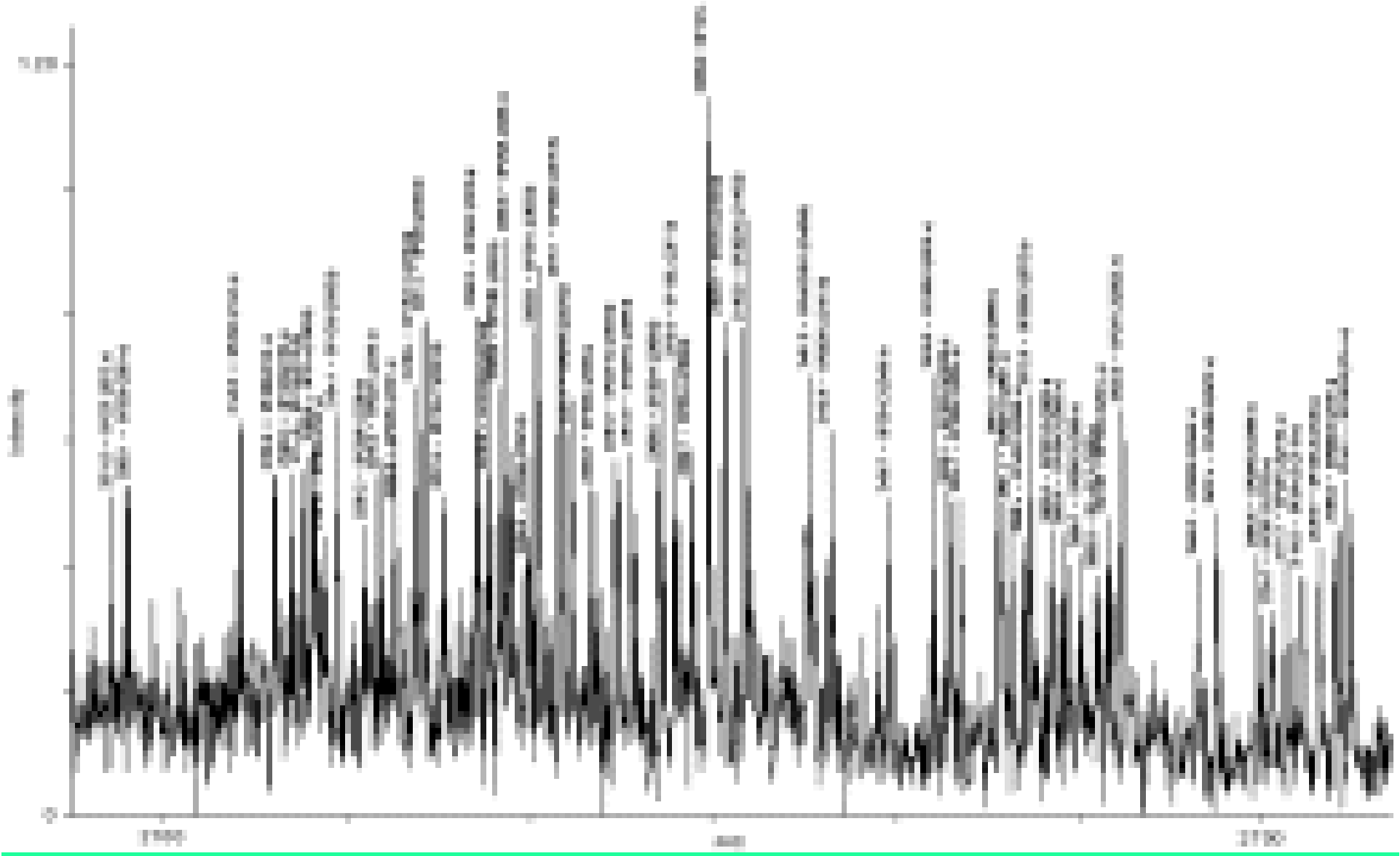
IMAGING?

RHEOLOGY

MALDI TOF

Matrix Assisted Laser Desorption Ionisation - Time Of Flight







TOM ORTEL
DUKE UNIVERSITY
MEDICAL CENTER,
DURHAM, NC

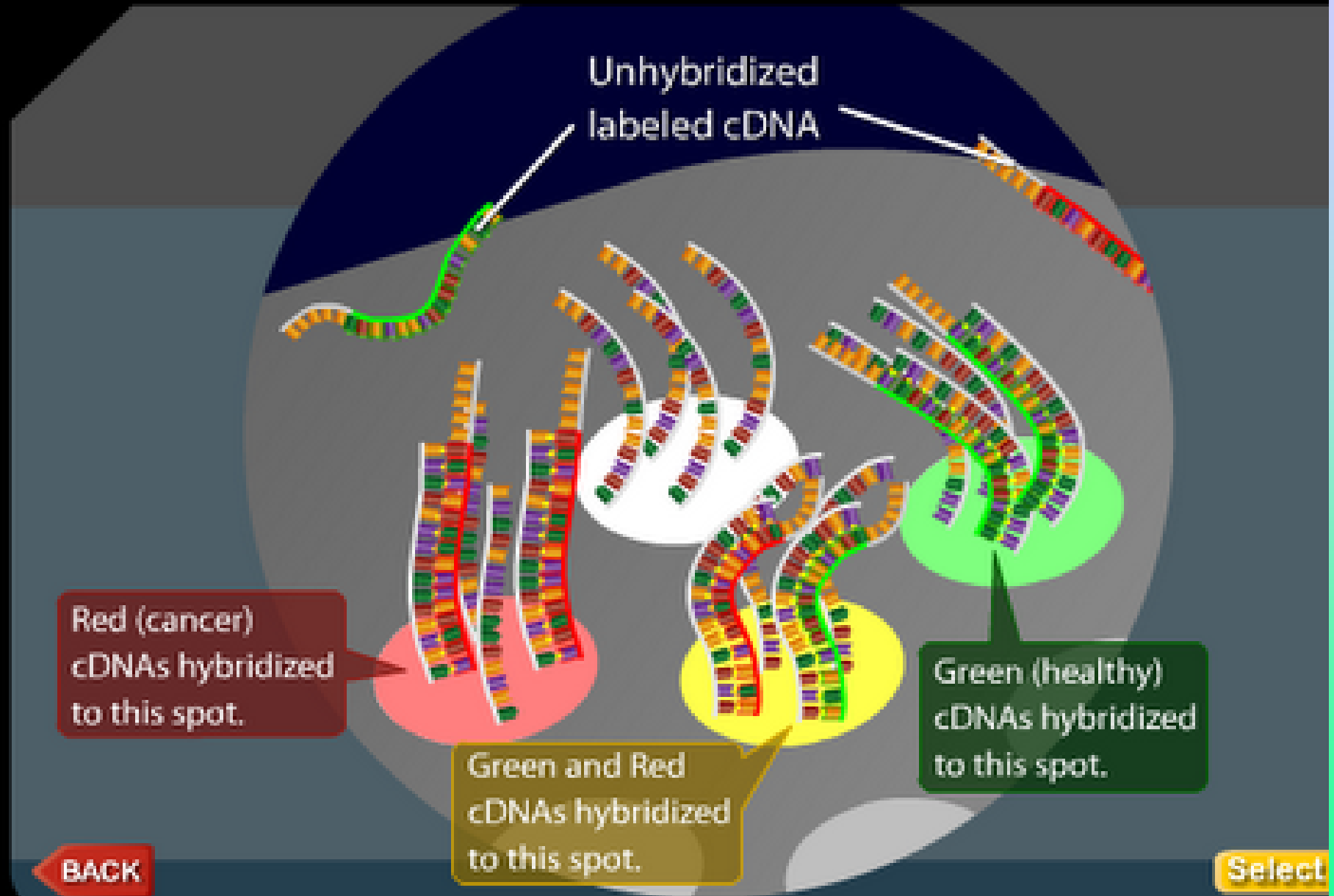
CONTRIBUTION FROM PROF. STEPHAN
MOLL, UNC SCHOOL OF MEDICINE,
CHAPEL HILL, NC.

1. Global hemostasis assays to predict thrombotic risk.
2. Monitoring tests for new oral anticoagulants.
3. Antithrombin gene sequencing to diagnose subtypes.
4. Antithrombin activity test without heparin.
5. Protein S and C gene sequencing to diagnose subtypes.

Gene-expression patterns predict phenotypes of immune-mediated thrombosis

- Potti A, Bild A, Dressman KK, et al. Blood 2006; 107; 1391-6. Duke University, Durham, USA.
- Studied patterns of gene expression in Anti-Phospholipid Syndrome (APS) – Total 129 patients:
 - 57 with APS and VTE (Venous Thromboembolism)
 - 32 no APS with VTE
 - 32 with APS no VTE
 - 8 no APS no VTE (normals)
- Used amplified RNA from peripheral blood cells and assessed patterns on DNA microarray chips (Affymetrix).

DNA MICROARRAYS



BACK

Select

1. Collect Tissue

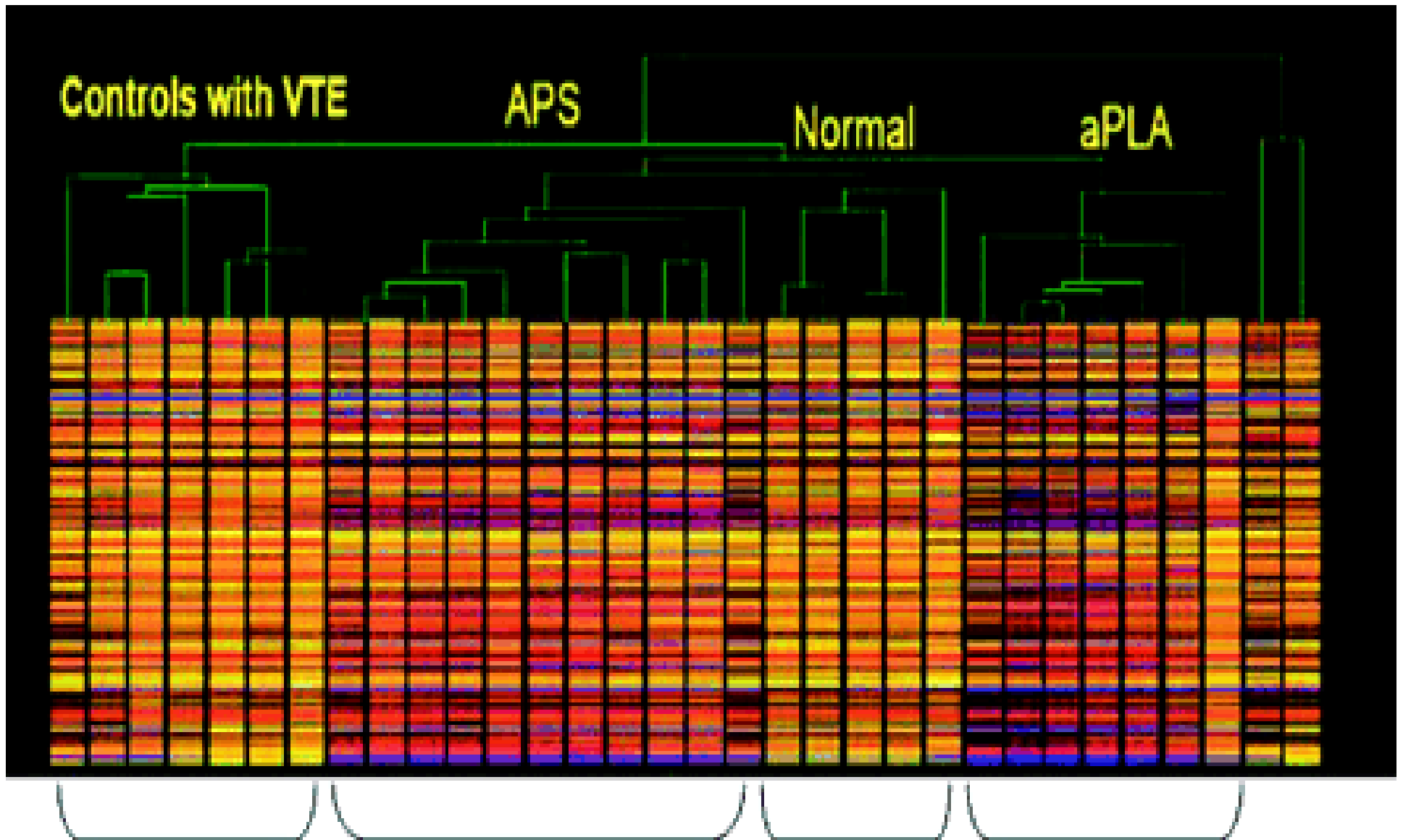
2. Isolate RNA

3. Isolate cDNA

4. Make Labeled DNA Copy

5. Apply DNA

6. Scan



Each horizontal line represents a single gene. Each column, a single patient.
Red=strongly upregulated
Blue=down regulated

RESULTS

- 106 genes were found to differ 2x between APS patients and non APS patients with thrombosis.
- These included regulators of apoH, factor X, thromboxane, zinc finger proteins, MMP's and interleukin 22.
- Followup study on “Unique whole blood gene expression profiles distinguish different clinical phenotypes of venous thromboembolism”
- Ie. DVT vs PE at Boston ISTH meeting 2009,
- “Transcriptome” analysis is coming!

TAKE UP OF NEW TESTS

- DRVVT for LA; 10 years.
- APC resistance; 3 years
- FDP-D-dimer; 15 years.
- Collagen binding assay for VWF; 20yr
- ProC global?: >10 years
- Platelet function analyser; 5 years
- XACT (for procoagulant phospholipid)???

THE FUTURE ?

- TRICORDER FROM STAR TREK



“HAEMOSTASIS” MODE
Remote sensing!
Monitors all known blood
factors and cells.

Detects compromised
endothelium.

Automatically applies
remedy?.