New Concept for Blood Coagulation

Magalie Faivre[1], Philippe Peltié[2], Anne Planat-Chrétien[2], Marie-Line Cosnier[1], Myriam Cubizolles[1], Christophe Nougier[3], Claude Négrier[3] and Patrick Pouteau[1]

1 CEA/LETI/DTBS/SBSC, 17 rue des Martyrs, 38054 Grenoble Cedex 9 France
2 CEA/LETI/DTBS/STD/LISA, 17 rue des Martyrs, 38054 Grenoble Cedex 9 France
3 Hospices Civils de Lyon, Hôpital Edouard Herriot, 5 place d'Arsonval, 69437 Lyon France

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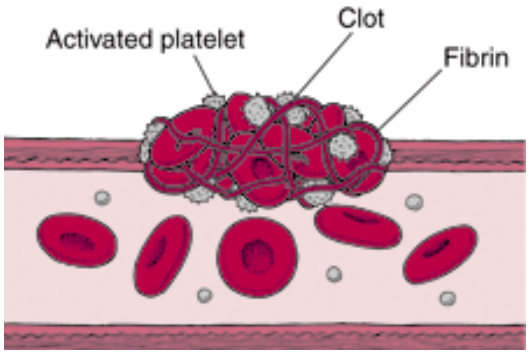
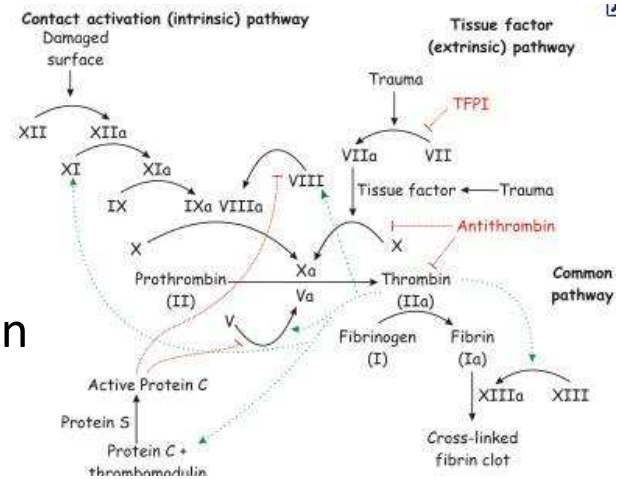
Introduction



- Coagulation
  - Important part of the haemostasis process
  - Clot = platelets + fibrin
  - Complex series of enzymatic reactions (proteins = coagulation factors)



- Coagulation studies
  - Disorders in coagulation factors
  - Prothrombin Time (PT), Activated Partial Thromboplastin Time (aPTT), Activated Cephalin clotting Time (ACT)

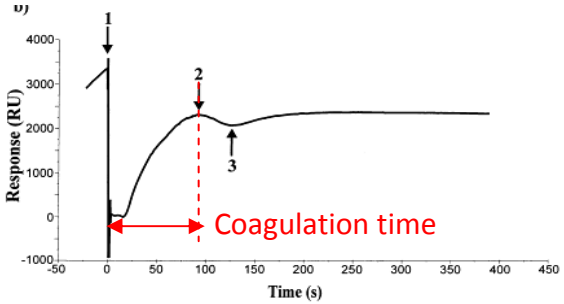
- Coagulation disorders
  - Increase risk of haemorrhage or thrombosis
  - Nearly 4 millions people are under oral anticoagulant therapy in Europe => daily monitoring of coagulation time => self adjust dosage of treatment

State of the art (1/3)

Many changes => Many techniques

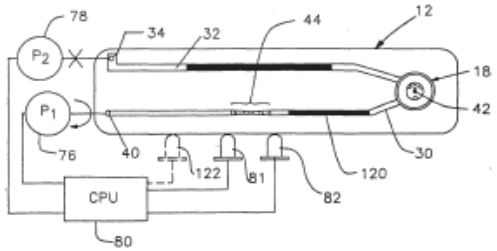
- R&D principles
  - Viscosity

SPR => reflected light intensity variations

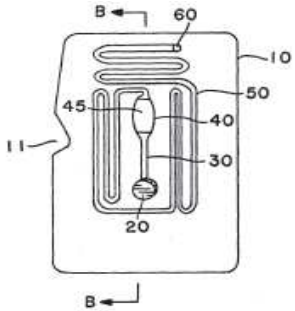


Hansson et al.,Biosensors & Bioelectronics, 1999

Capillary migration => migration time variations

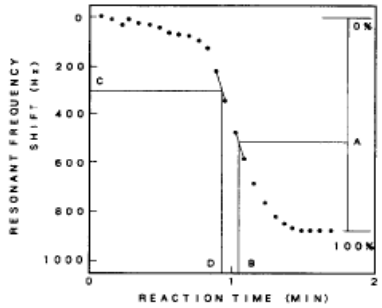


Cusack et al., 1994, US5302348



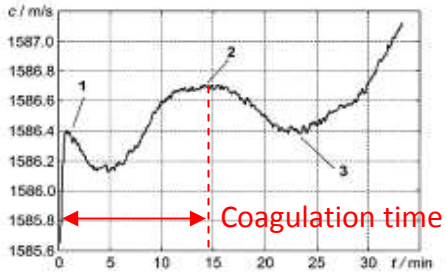
McDonald et al., 1991, US5039617

QCM => resonance frequency variations



Muramatsu et al.,Biosensors & Bioelectronics, 1991

US => propagation speed variations

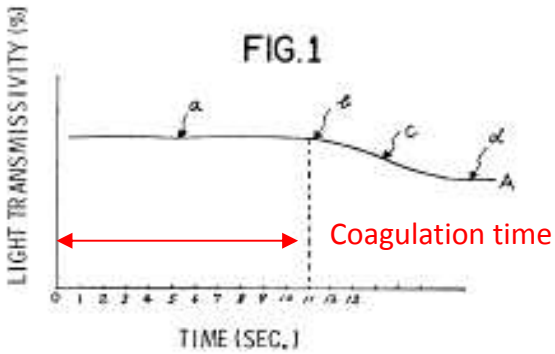


Voleišis et al., Ultrasonics, 2002

State of the art (2/3)

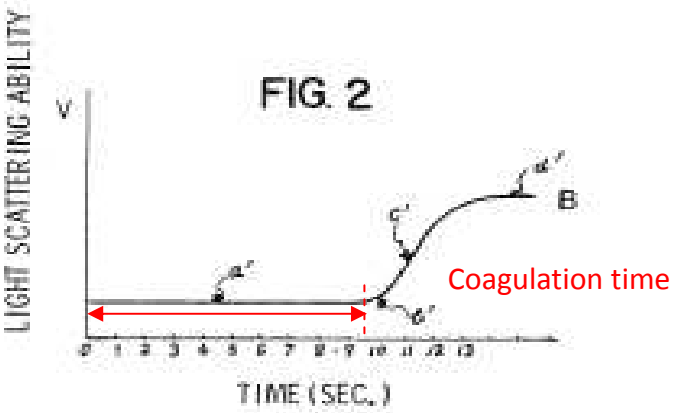
- Optical properties

Transmission => Decrease if transmitting light intensity



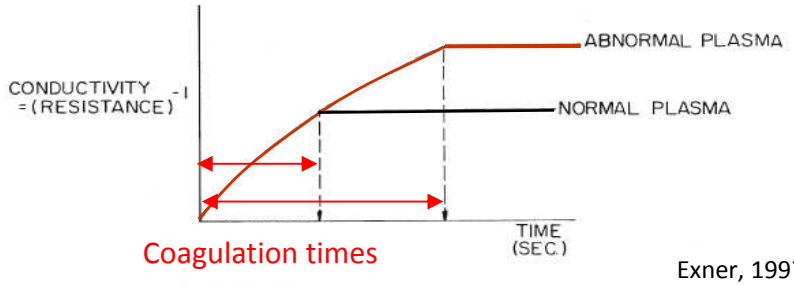
Kishimoto et al., 1981, US422538

Diffusion => Increase if the perpendicularly diffracting light



Kishimoto et al., 1981, US4252538

- Electrical properties



Exner, 1997, US5601995

=> Diversity of existing methods under development

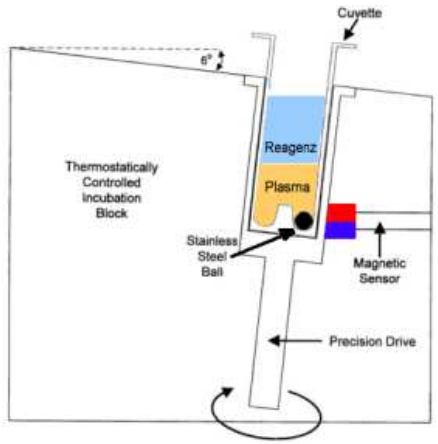
State of the art (3/3)

- Historical lab methods
  - "Tilt tube" method => tilt back a tube
  - "Wire loop" method => dip a wire loop

} Very manual

- Currently used principles in labs equipment

Immobilization of a moving probe



Changes in optical density

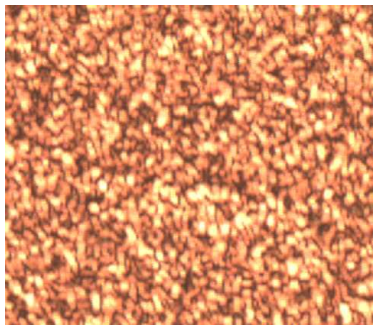
New concept for blood coagulation measurement

- Detection of blood cells immobilization => simpler and faster
- Prothrombin Time (PT): time for citrated plasma to clot in presence of tissue thromboplastin and $CaCl_2$ at 37°C
- Standardization by using INR

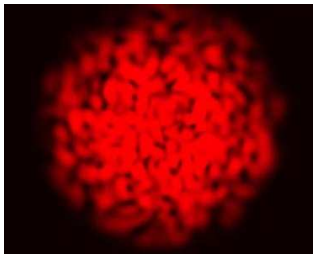
  $1 < INR < 7$

$$INR = \left(\frac{PT_{patient}}{PT_{reference}} \right)^{ISI}$$

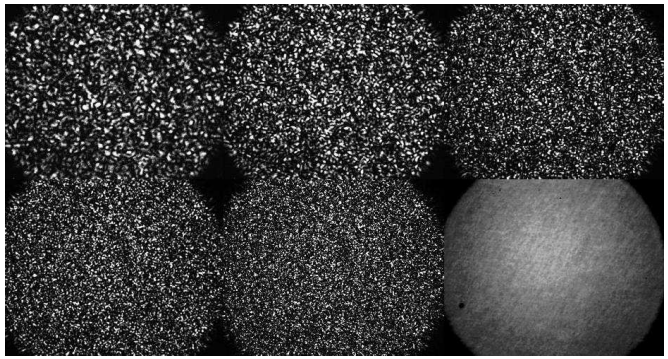
- **Multiple scattering dynamics** = observation of speckle patterns
  - Illumination of diffusing objects with laser
  - Constructive and destructive interferences => speckle pattern
  - Spot size related to optical system



1



2 - Vadim Makarov, Norwegian University of Science and Technology

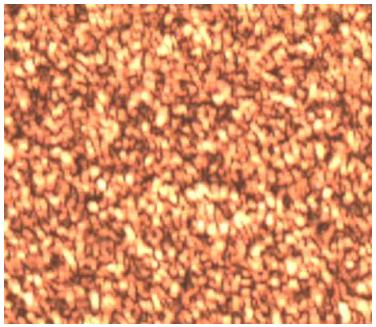


3 – http://luxrerum.icmm.csic.es

New concept for blood coagulation measurement

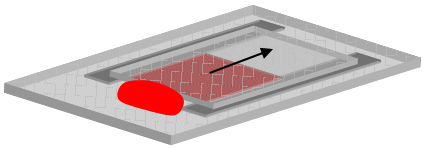
- Movement of blood cells => “swarming like” behavior
- Immobilization of cells => fix behavior => coagulation

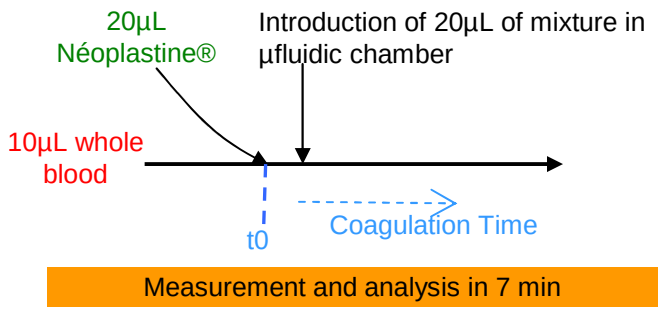
- Detection of immobilization in comparing patterns one by one
- When patterns identical => coagulation



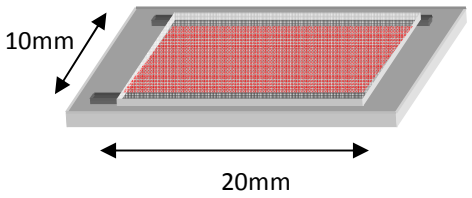
Experimental (1/3)

- Blood samples in tubes from **EFS** and **Edouard Herriot Hospital**
- Simple protocol at RT:





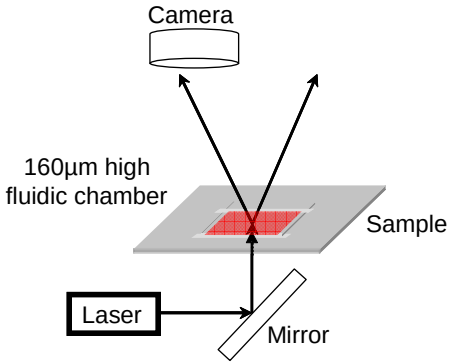
- Size



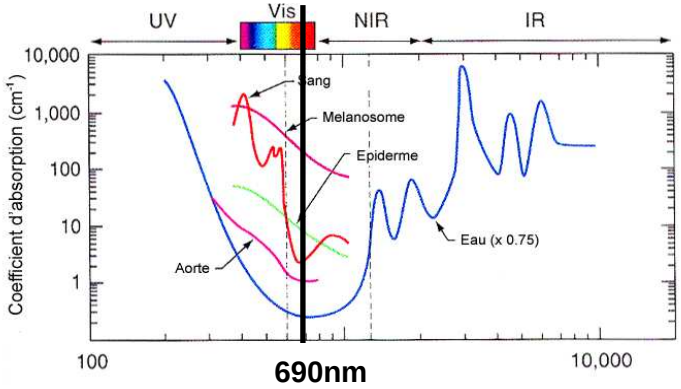
- Thickness : 160µm
  - ⇒ Speckle well defined
  - ⇒ Blood absorption

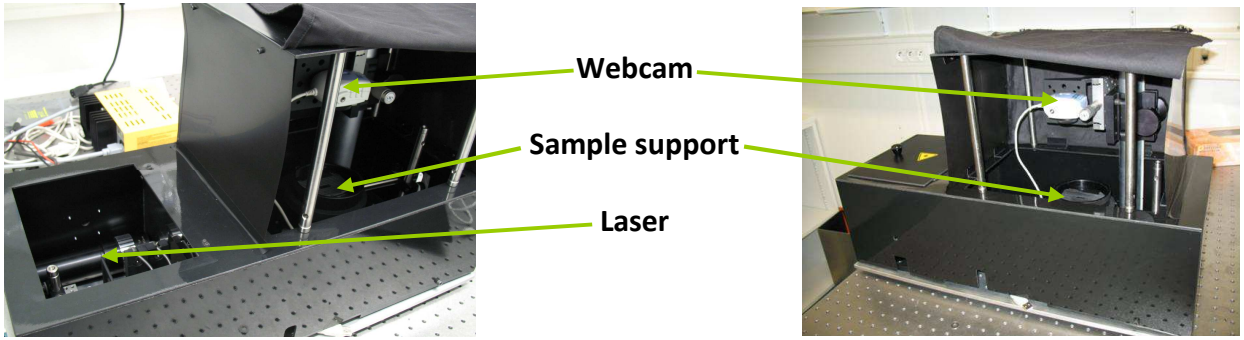
Experimental (2/3)

- Optical set-up
  - Laser: 10 to 20mW, 690nm



Why 690nm?





Maximizing signal-to-noise ratio => Adequacy between detector and optical system

Experimental (3/3)

- Acquisition
  - T0 = triggered on the laptop simultaneously with mixing
  - Rate = 1fr/s for 4 minutes

  $\Rightarrow$ Movie

- Images analysis
  - Consecutive images comparison with correlation coefficient

$$R = \frac{\sum_{i=1}^{N}(I_i - \bar{I}) \times (J_i - \bar{J})}{\sqrt{\sum_{i=1}^{N}(I_i - \bar{I})^2} \times \sqrt{\sum_{i=1}^{N}(J_i - \bar{J})^2}}$$

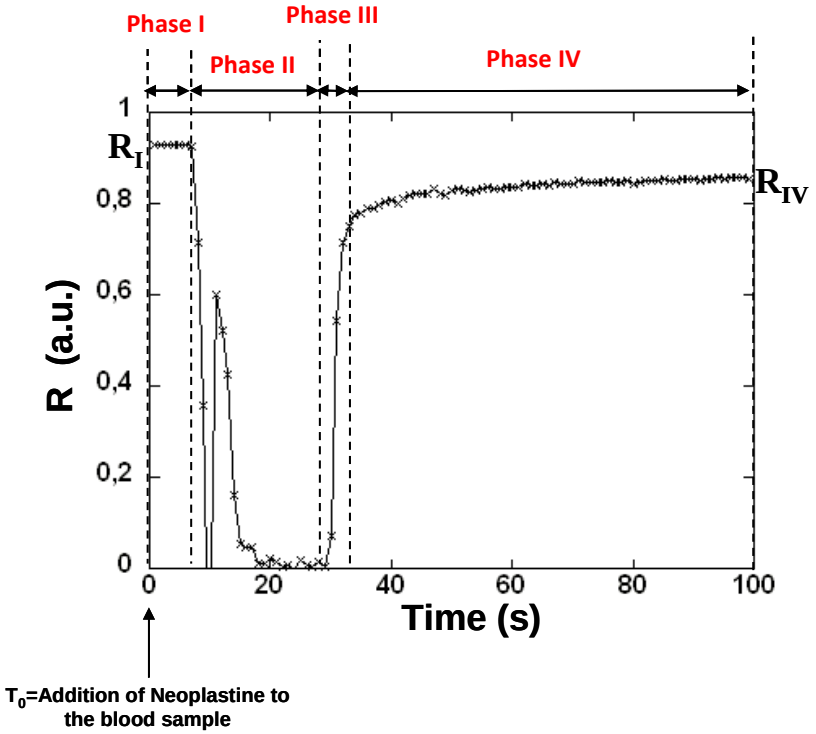
N = number of pixels
Ii and Ji = pixel intensity of a picture and the previous or following
$\bar{I}$ and $\bar{J}$ = mean intensity for pictures I and J respectively

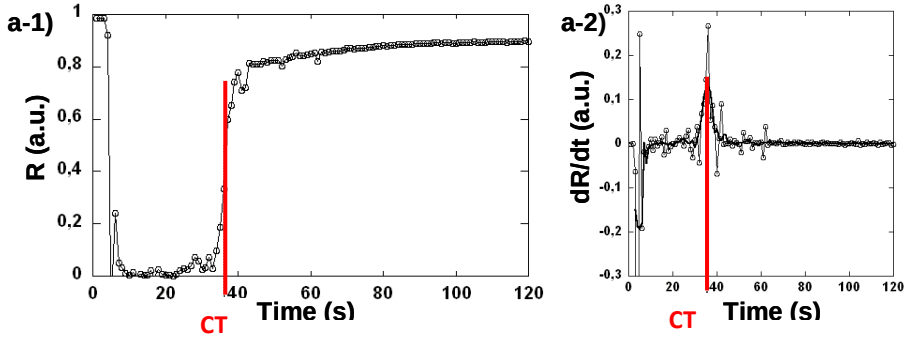
  - Derivative

$$\frac{dR}{dt} = f(t)$$

$\Rightarrow$ **Maximum of f(t) = coagulation time**

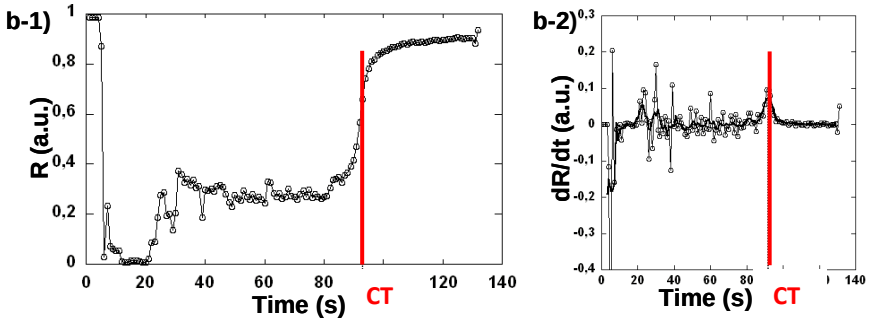
Results (1/4)





Normal curve

PT = 37s



Pathological curve

PT = 91s

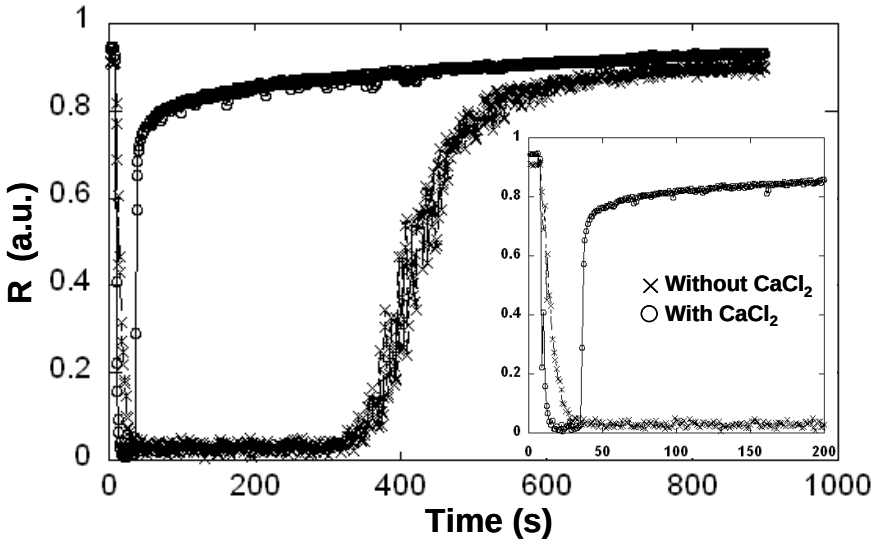
⇒ **CV = 4.5%**

⇒ **Good repeatability**

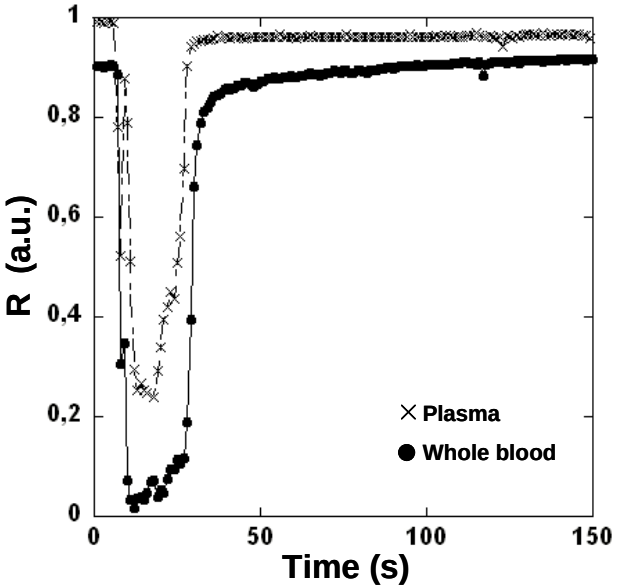
Results (2/4)

- Control experiments

Without $CaCl_2$



On plasma



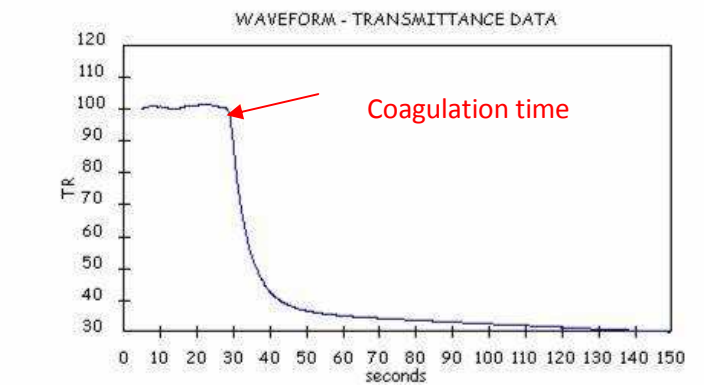
- PT with $CaCl_2$= 37s
- No PT without $CaCl_2$
- Sedimentation

- PT on whole blood = 31s
- PT on plasma = 29s
- Larger dynamical variation for whole blood

⇒ Detection of coagulation

Results (3/4)

- Reference method = Edouard Herriot Hospital, Lyon Laboratory of hematology
  - MDA II by Trinity Biotech



	Reference method	Multiple scattering method
Principle of measurement	transmittance	speckle correlation
Sample volume	50µL of plasma	10µL of whole blood
Temperature	37°C	room temperature
Replicate	1	3
Range	10-60s	25-150s
Duration of measurement	20min centrifugation + 5min analysis = 25min	7min
Repeatability	CV < 5%	CV < 10%

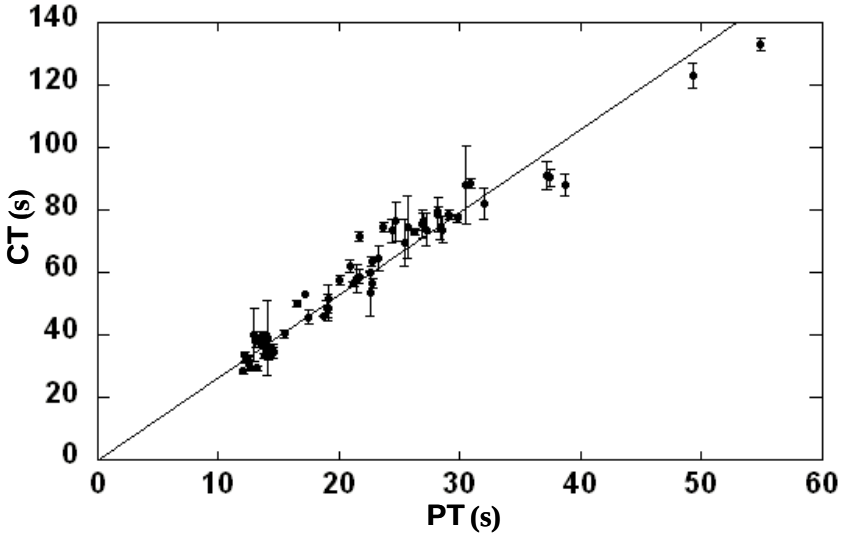
⇒ **Temperature**

⇒ **Duration**

Results (4/4)

- Comparison with reference method (65 patients)

PT measurements



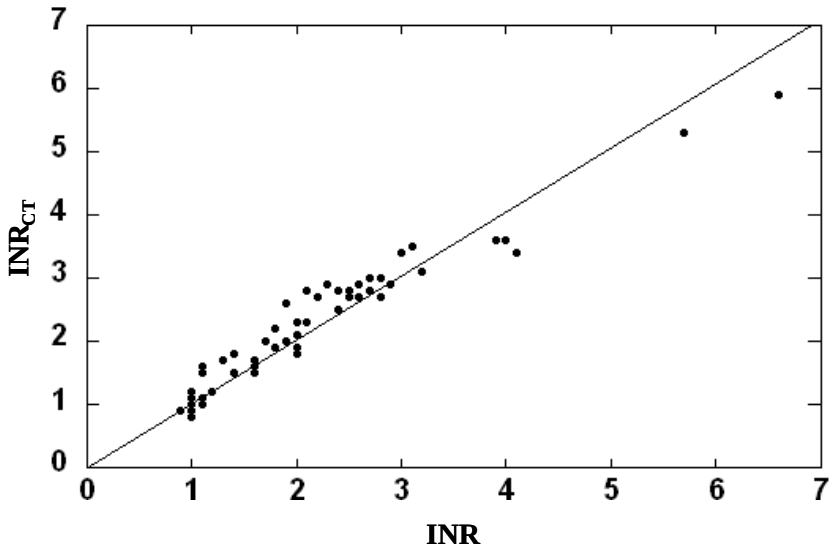
$R^2 = 0.94$

Y = 2.6491x

T° difference => PT lower

CV < 10%

INR



$R^2 = 0.93$

y = 1,0159x

⇒ **Determination of INR with good sensitivity**

Conclusion

- Novel method of determining coagulation time based on analysis of the resulting speckle “swarming-like” behavior
- **Benefits:**
  - **Simple**
    - **On whole blood (no plasma separation)**
    - **Simple optical set-up**
  - **Accurate (sudden phenomenon)**
  - **Very good agreement with reference measurements**
  - **Good repeatability (cv < 10%)**
- Applicable to any coagulation times (Activated Cephalin clotting Time (ACT) or Activated Kaolin clotting Time (AKT))

- Available online : **Coagulation dynamics of a blood sample by multiple scattering analysis**. Journal of Biomedical Optics 2011 May; 16(5): 057001
- US 20100248278 « **Method and a device for characterizing the coagulation or sedimentation dynamics of a fluid such as blood or blood plasma** », published Sep. 30, 2010

Thank you for your attention

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LABORATOIRE D'ÉLECTRONIQUE
ET DE TECHNOLOGIES
DE L'INFORMATION

CEA-Leti
MINATEC Campus, 17 rue des Martyrs
38054 GRENOBLE Cedex 9
Tel. +33 4 38 78 36 25

www.leti.fr

cea

MINATEC

INSTITUT CARNOT CEA LETI