

# **KILLING THROMBUS WITH NANO BULLETS**



**D. Dash**

***Department of Biochemistry  
Institute of Medical Sciences  
Banaras Hindu University***

# Thrombus has two components:

(1) Protein Component

composed of

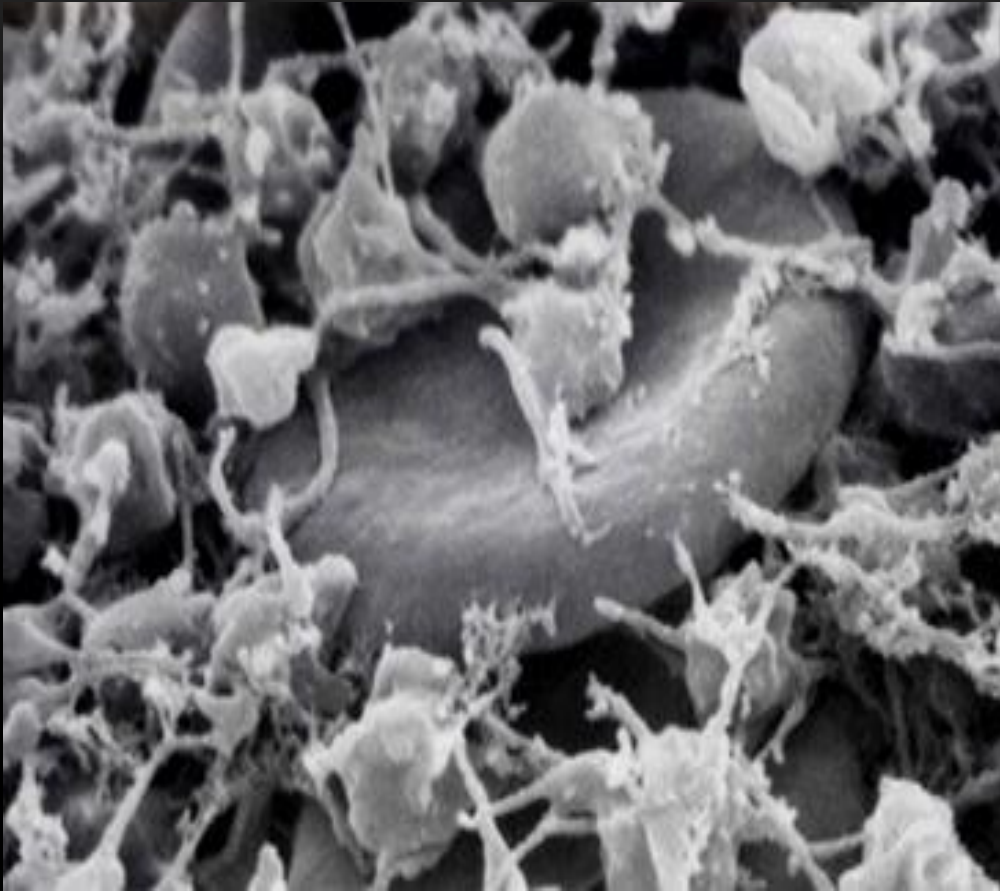
*Insoluble Fibrin Clot*

(2) Cellular Component

composed of

*Aggregated Platelets*

# Arterial Thrombosis



Scanning electron micrograph of thrombus  
(RBC entrapped within aggregated platelets)



# Two components of Anti-Thrombotic Measures:

## (1) Fibrinolytic Drugs

*(e.g. Streptokinase, Urokinase  
& Alteplase)*

## (2) Anti-Platelet Drugs

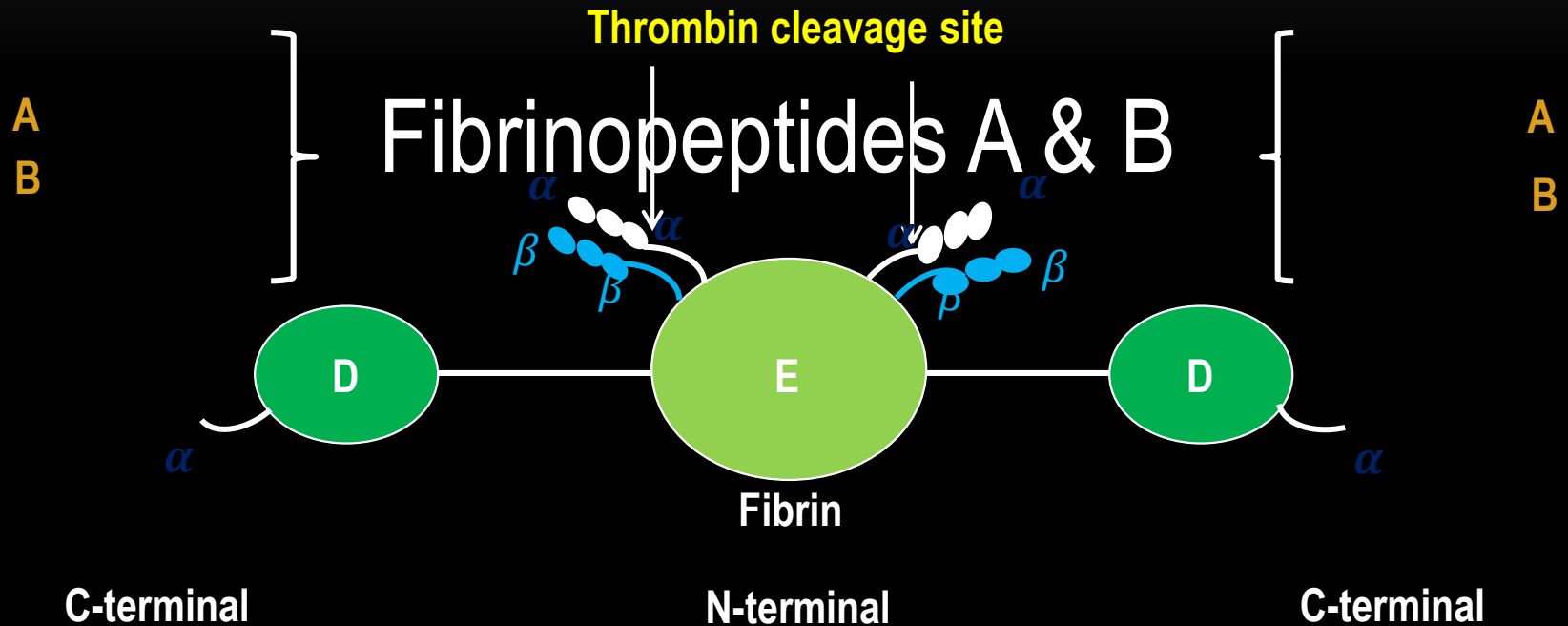
*(e.g. Aspirin, Clopidogrel  
& Ticagrelor)*

**Fibrinolytic therapy is associated  
with  
serious bleeding complications!  
(Off-Target Effects)**

**Requires consistent physician  
supervision & monitoring!**

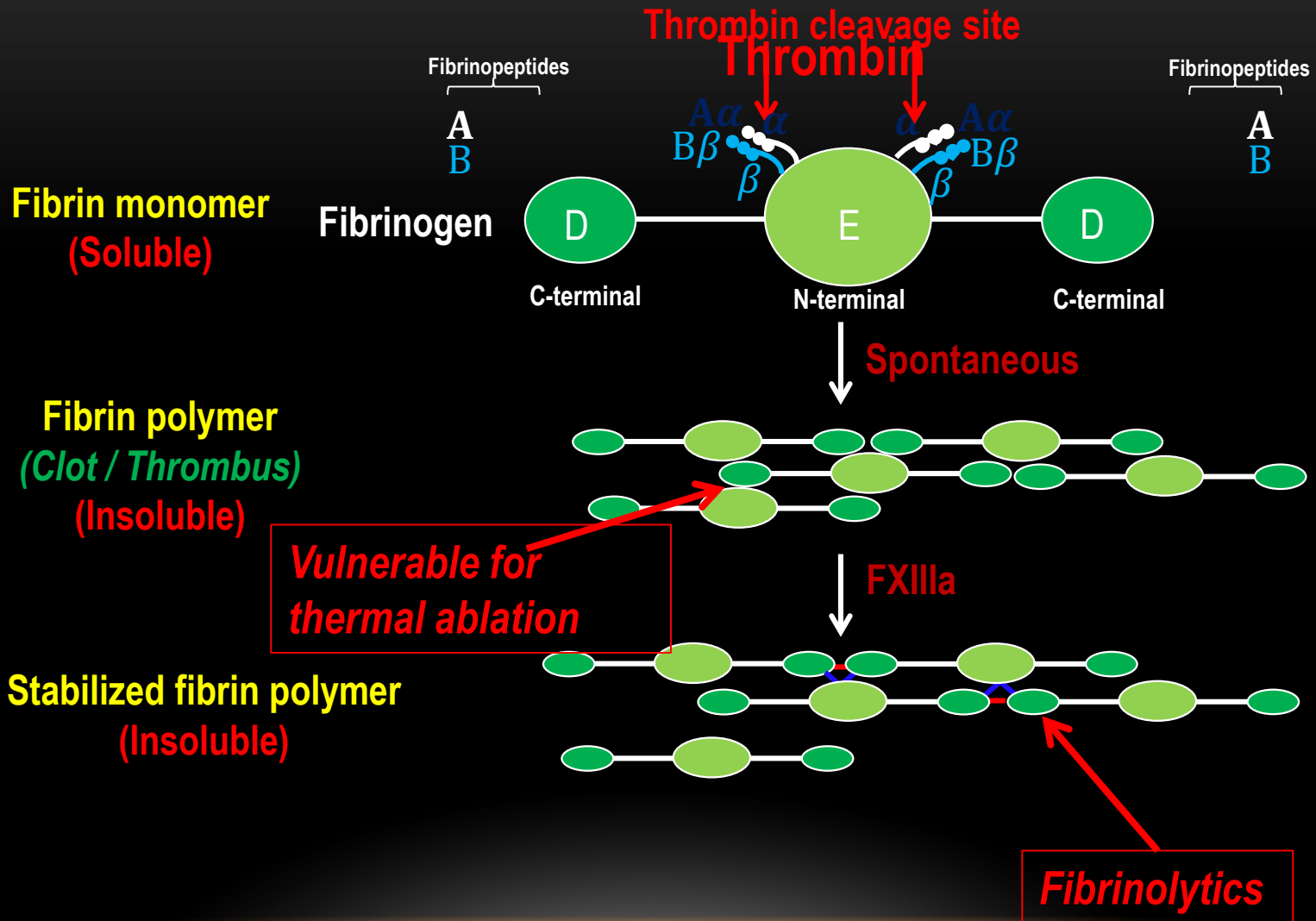
# FIBRINOGEN

Products of Thrombin cleavage site



# FIBRIN

# Formation of Fibrin polymer



# PHOTOTHERMAL THERAPY

*NIR LASER IRRADIATION  
(808 NM)*



**GOLD NANORODS / GRAPHENE**

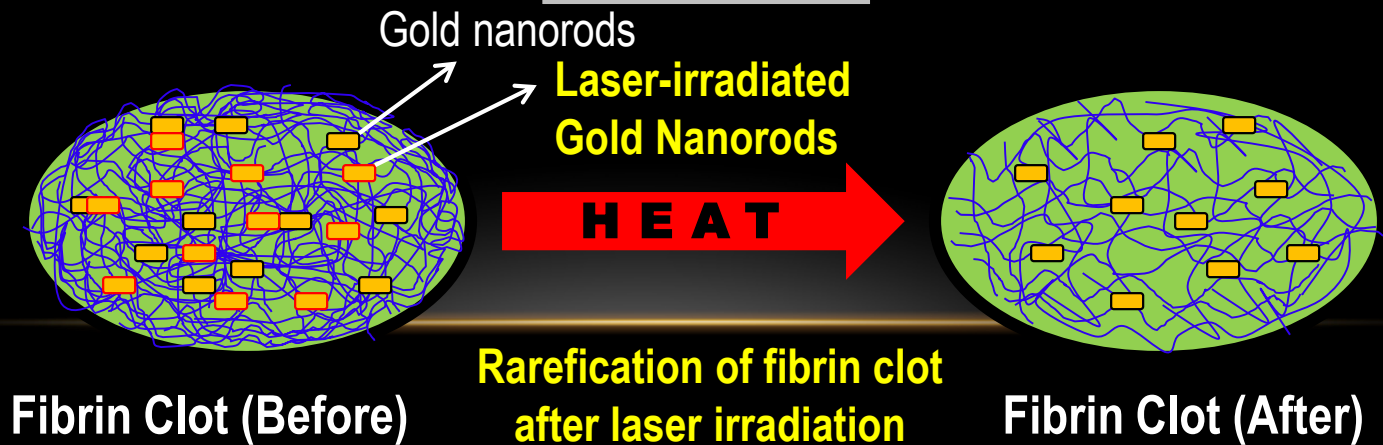
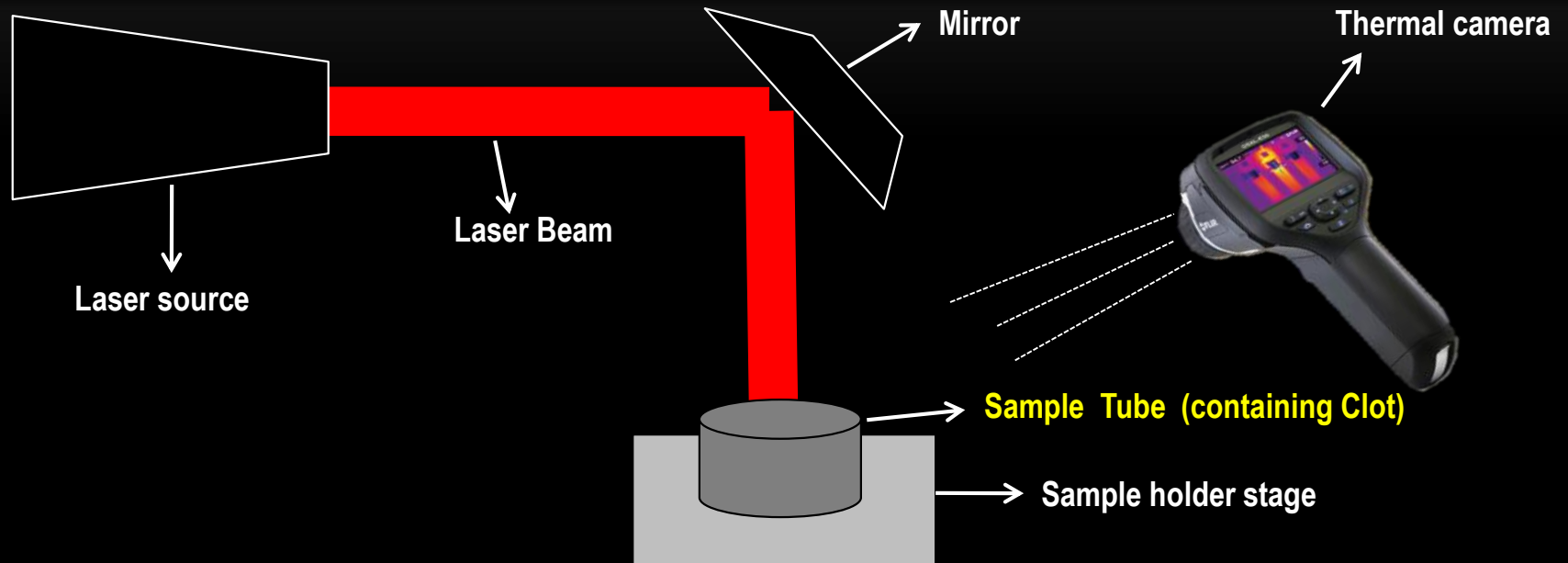


**HEAT**

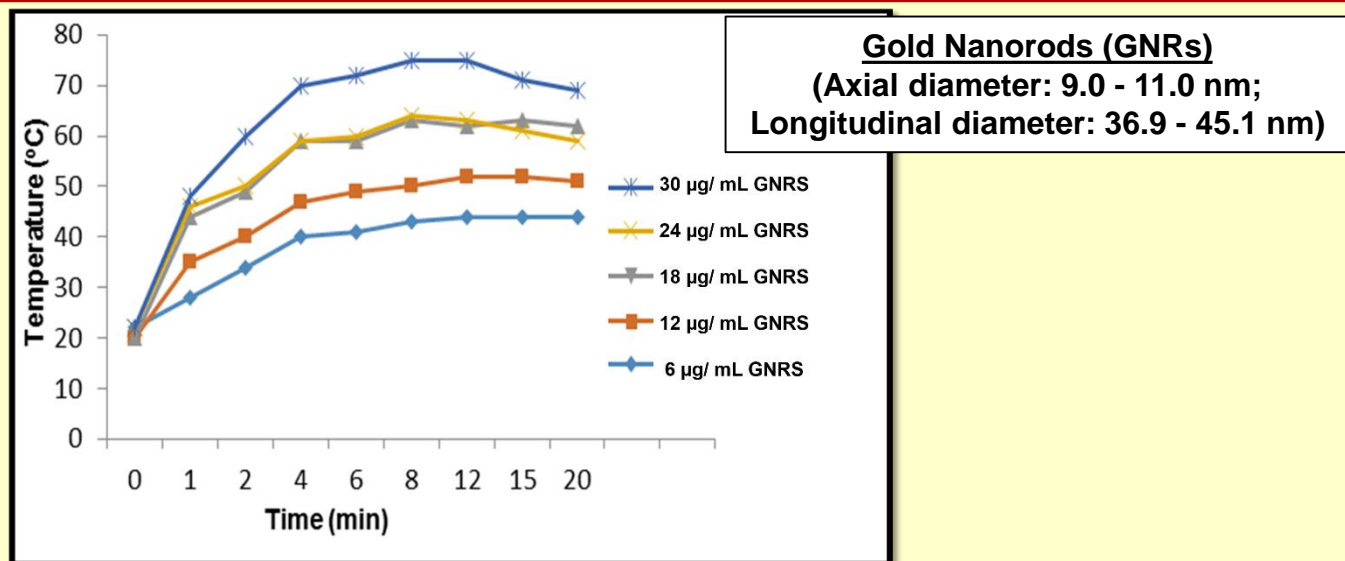
**DEATH OF CANCER CELLS**



# Set-up: *In vitro* Photothermal Abalation of Fibrin Clot



# RISE IN TEMPERATURE IS A FUNCTION OF GNR CONC.

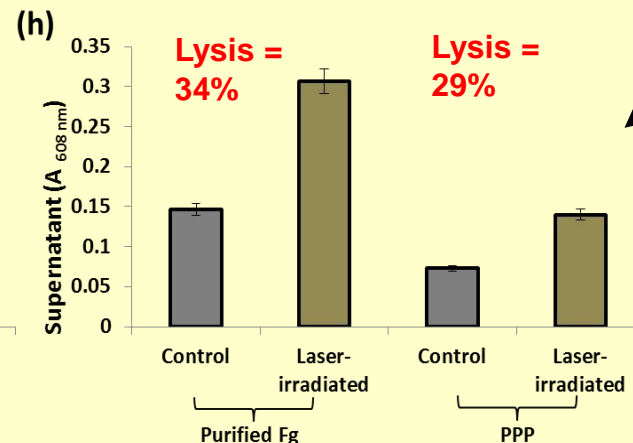
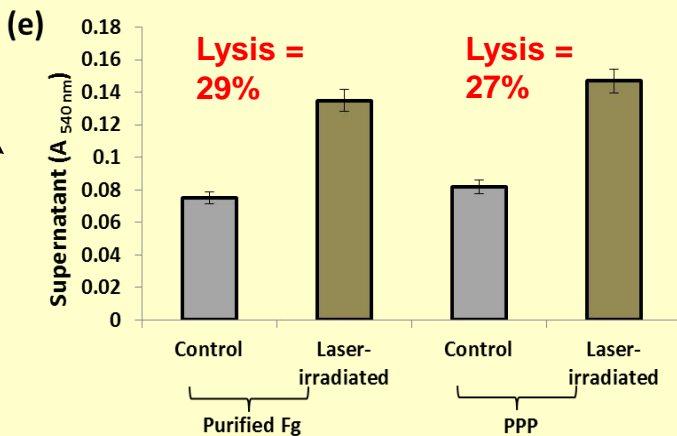
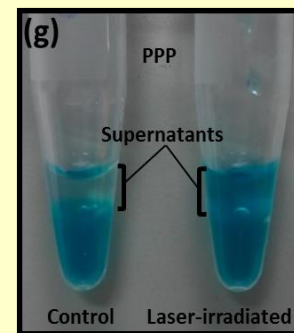
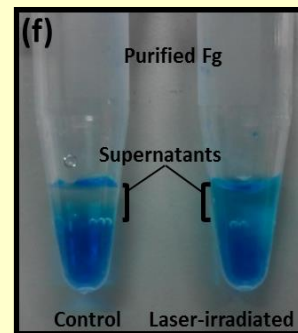
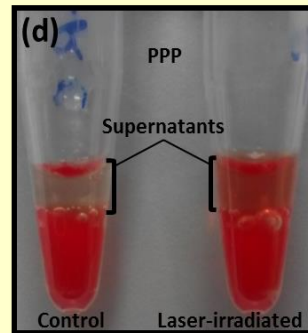
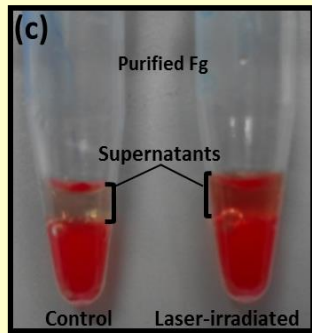
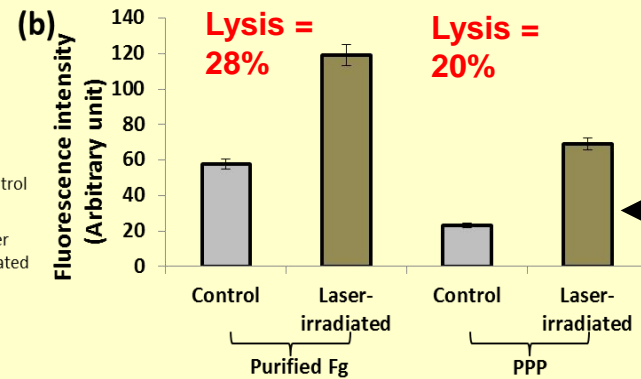
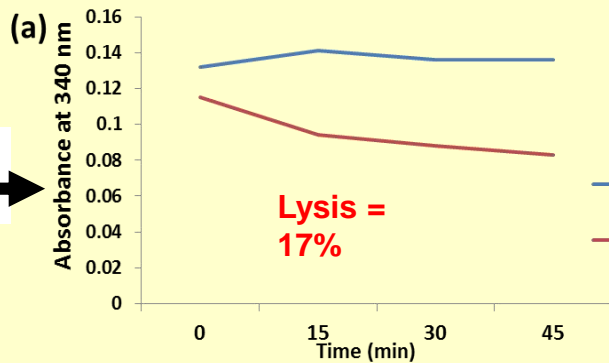


**RISE IN TEMPERATURE (°C) OF SOLUTION WITH INCREASE IN GNR CONCENTRATION**



Infra red (IR) thermal images of GNR samples in microplate wells (Left Panel) or centrifuge tube (Right Panel) exposed to the NIR laser at power density of 1.05 W/cm<sup>2</sup>. Within the panel cursor represents spot temperature and the vertical pseudo-color bar signifies temperature intensity from high (yellow) to low (dark blue).

# PHOTOTHERMAL ABLATION OF FIBRIN CLOT



Photothermal ablation of clot (a) Turbidity assay (purified Fg) (b) Fluorescence assay in both purified Fg and PPP (c) and (d) Drabkin's assay in purified Fg and PPP (e) corresponding histogram (f) and (g) Methylene Blue assay in both systems and (h) corresponding histogram. Each histogram is a representative of five different sets of experiments.

# PHOTOTHERMAL ABLATION OF THROMBUS UNDER FLUID SHEAR

**Venous Shear**  
**(500 s<sup>-1</sup>)**

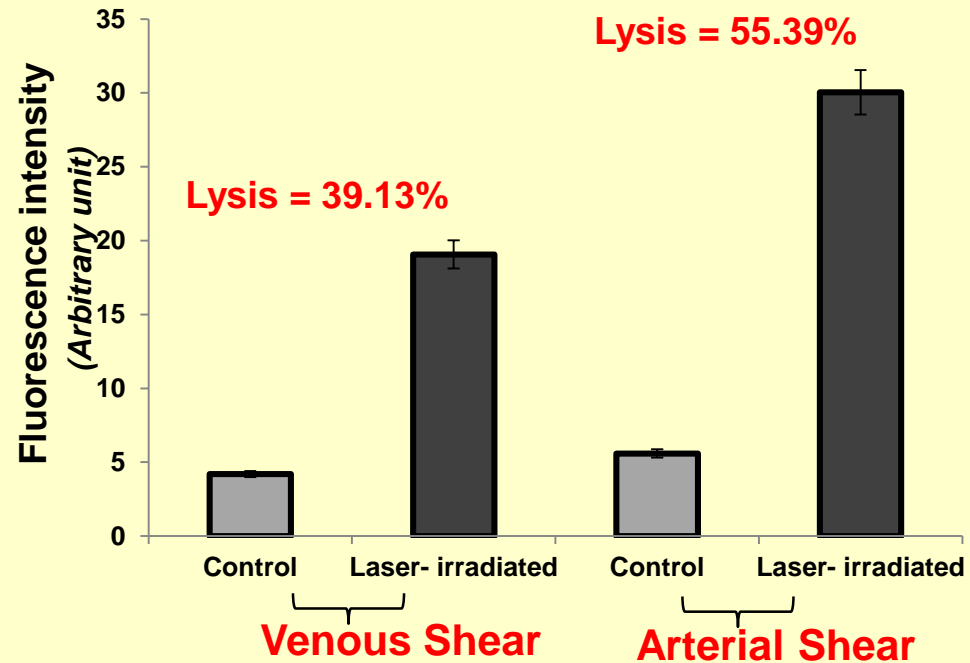
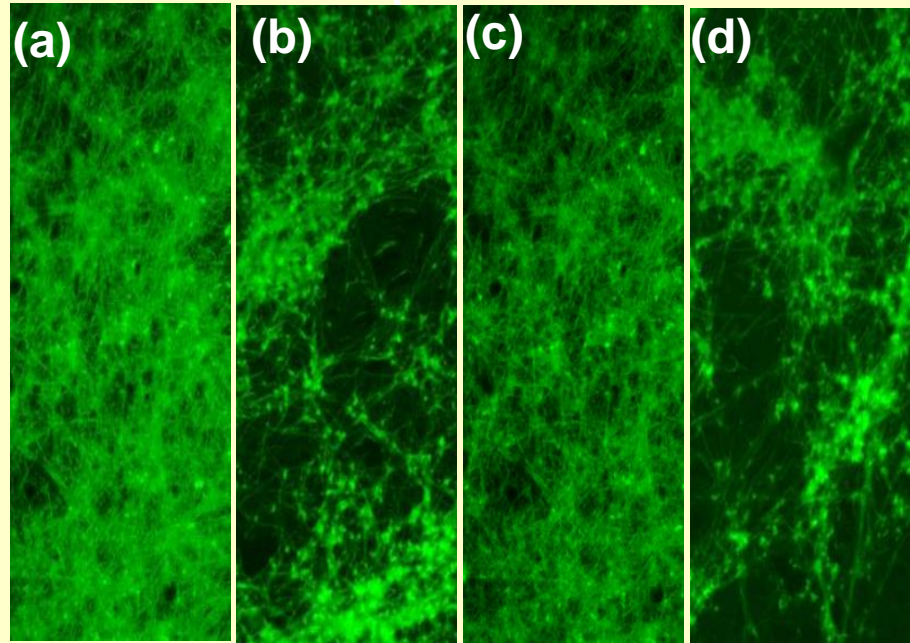
**Arterial Shear**  
**(1500 s<sup>-1</sup>)**

Control

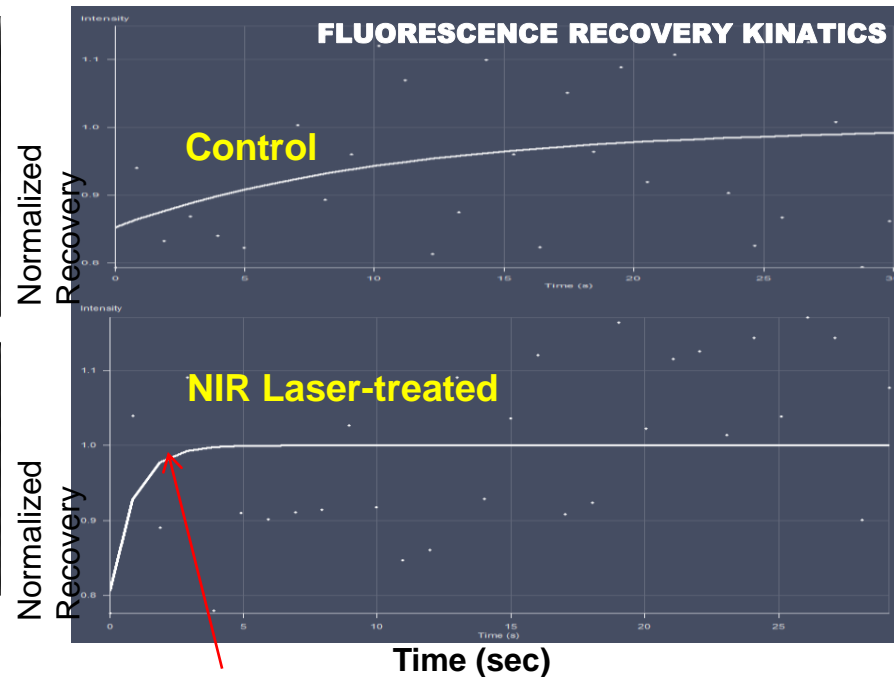
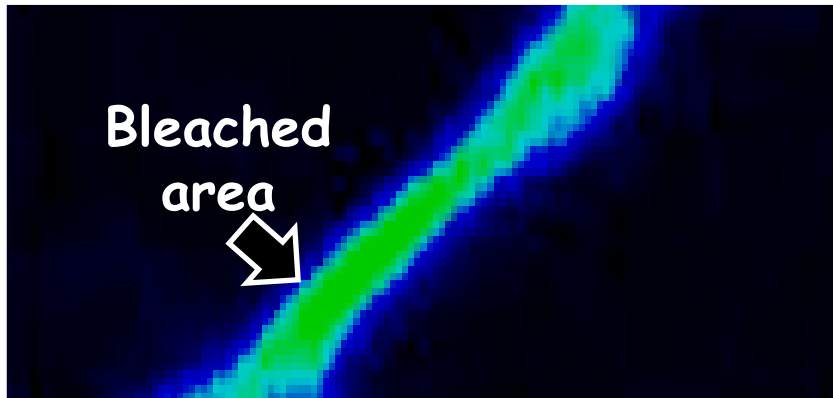
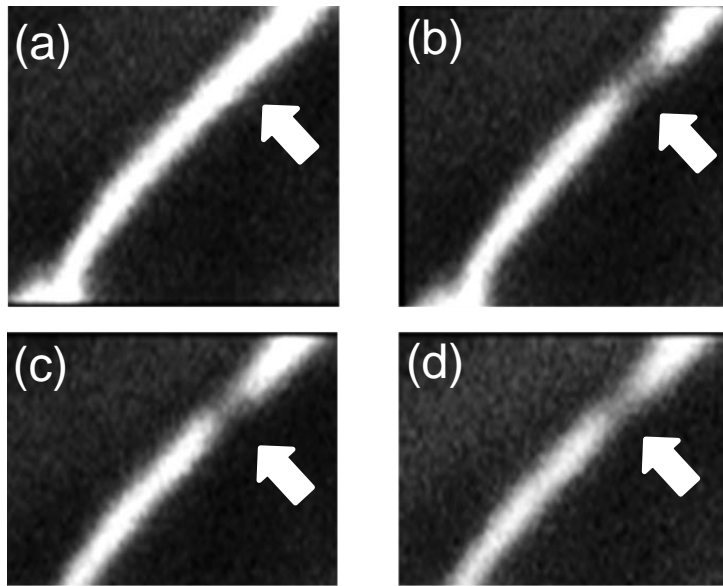
Laser

Control

Laser



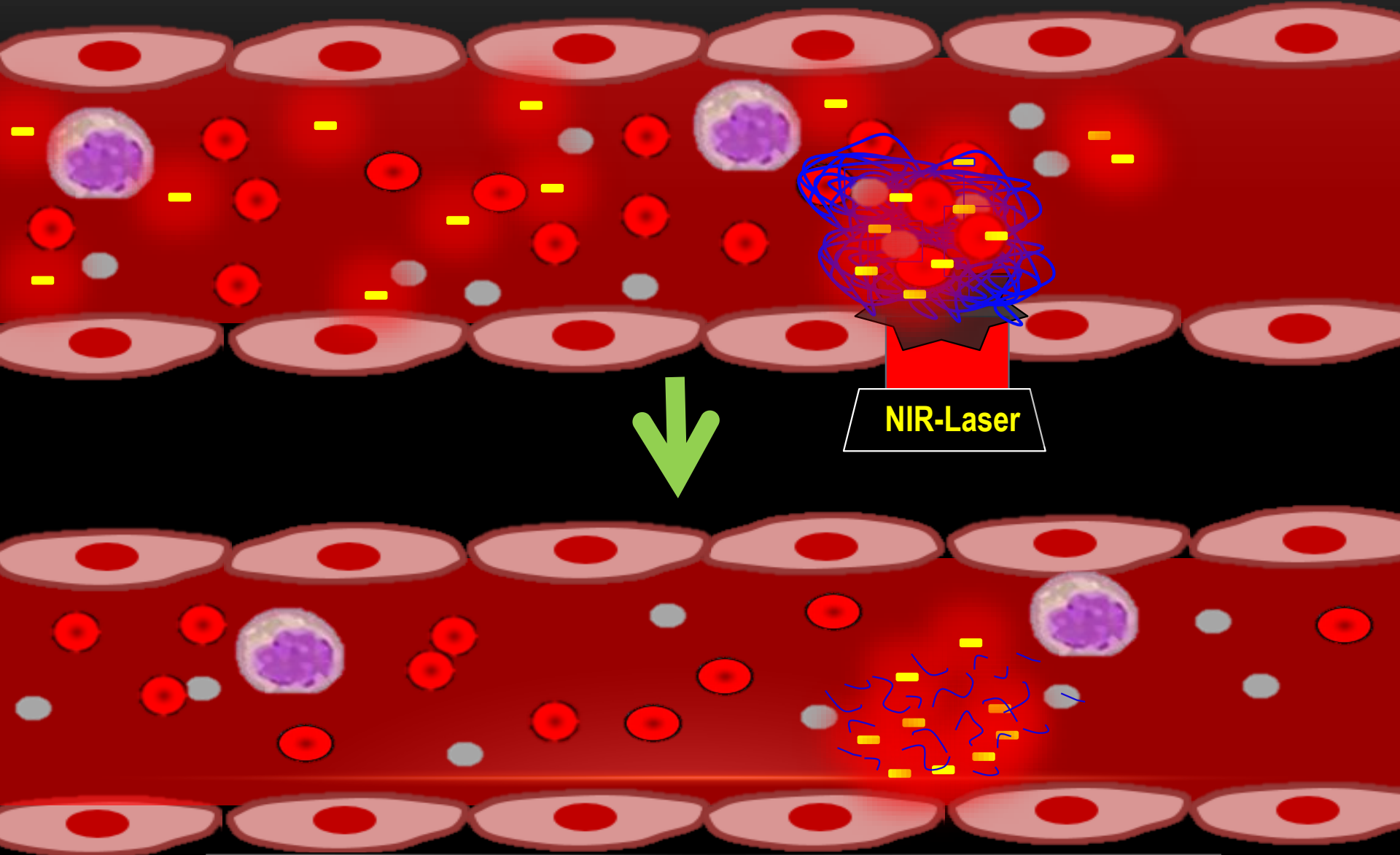
# FRAP (Fluorescence Recovery after Photobleaching) analysis by Confocal Microscopy



**Faster fluorescence recovery represents greater degree of molecular dynamism in laser-treated fibrin clot.**

FRAP analysis of 10% Alexa 488-labeled fibrin strands. (a) Before iteration, (b) photobleached with 100% laser (488 nm) power. (c and d) chronological events demonstrating fluorescence recovery at 2% of excitation laser power. White Arrows indicates the region of interest.

# Our Experimental Strategy of Thrombolysis



Separation of Fibrin Strands & Restoration of Blood Flow

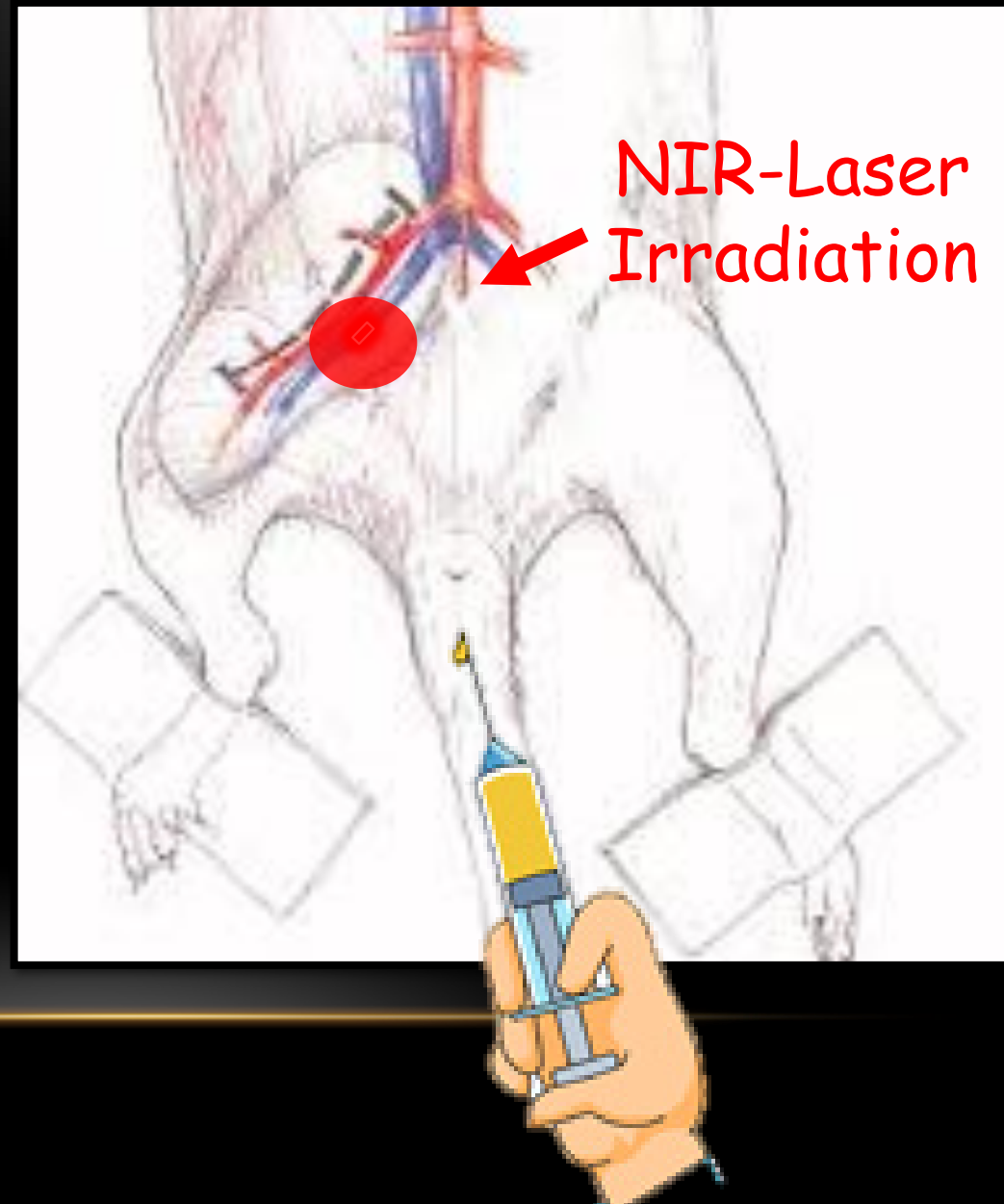


# Photothermal Lysis in Mouse Model

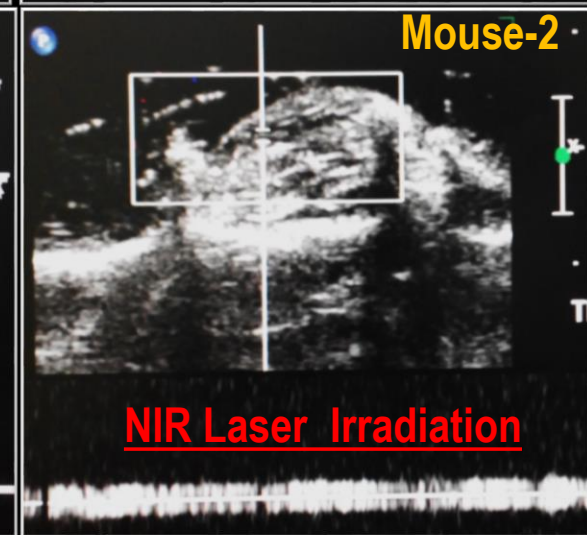
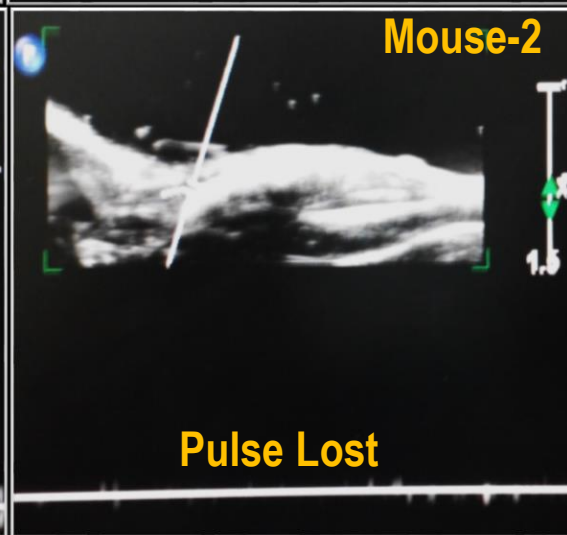
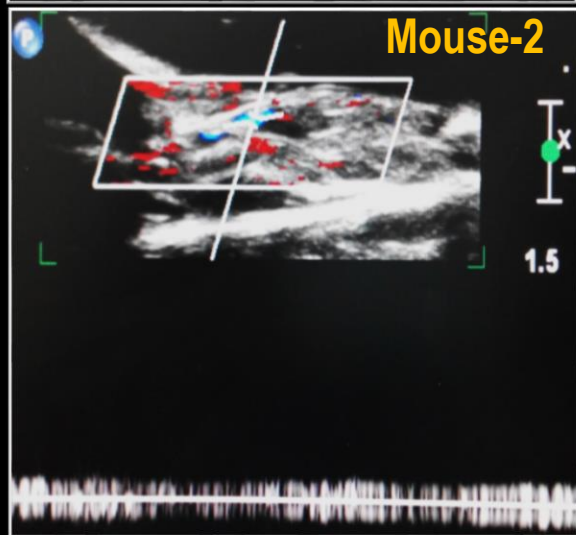
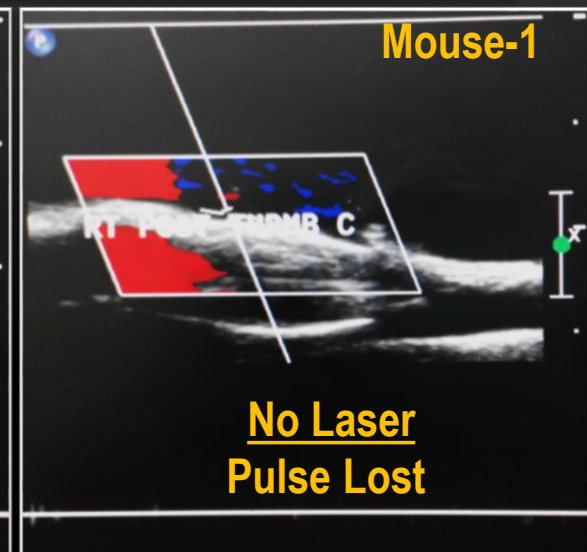
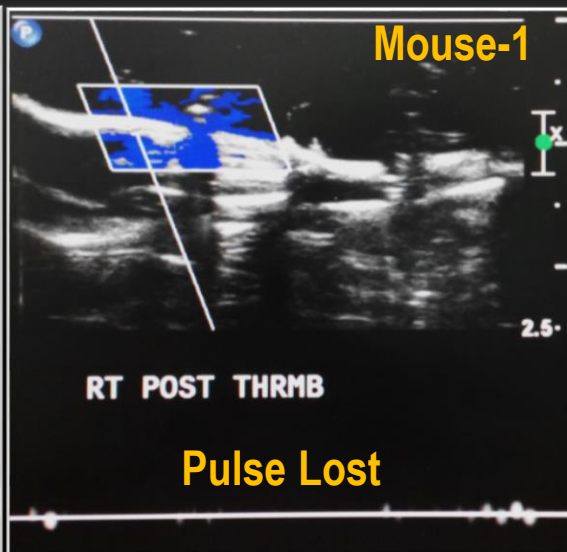
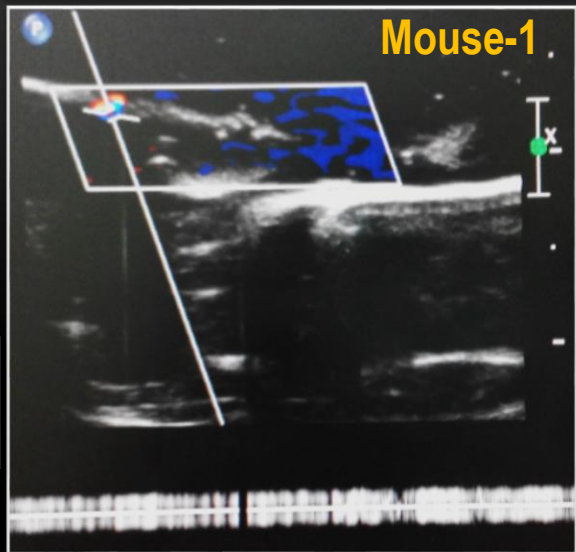
Step I- Thrombus induced in Femoral vein by  $\text{FeCl}_3$  injury.

Step II- Fibrin-targeted GNR injected through caudal vein.

Step III- Thrombus irradiated with NIR- laser.



# Colour Doppler Scan for Blood Flow in a Murine Femoral Vein



Doppler Scan before Clot Formation showing Routine Blood Flow

Doppler Scan after Clot Formation showing Occluded Blood Flow

Doppler Scan Showing Restoration of Blood Flow after Laser- Irradiation in Mouse 2



# Can *Low-Dose* Streptokinase Therapy

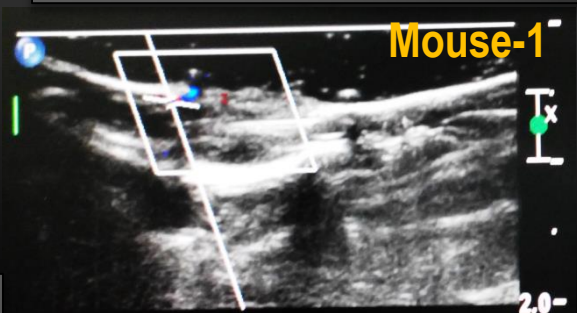
(*not* associated with bleeding complications)

*synergize*

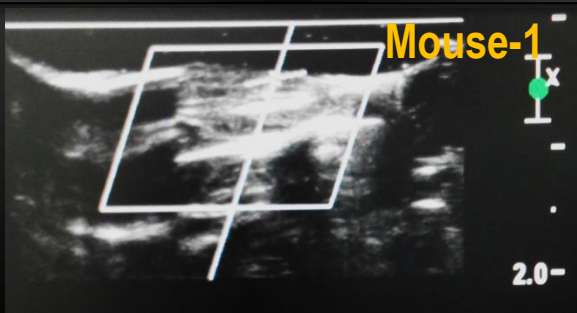
with Photothermal Therapy

Towards *effective Thrombolysis*?

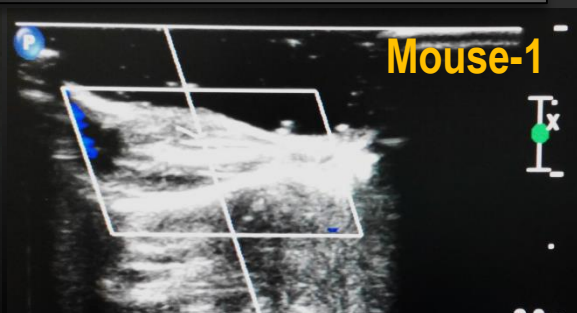
# Colour Doppler Scan for Blood Flow in a Murine Femoral Vein



pulse wave

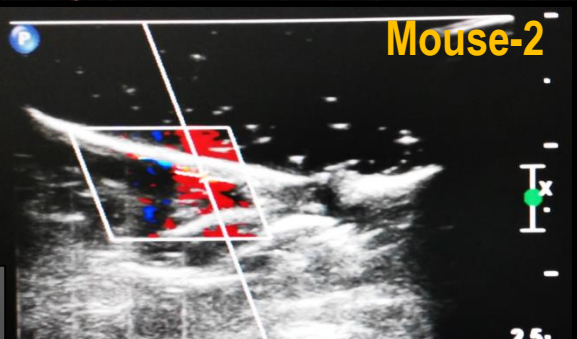


Pulse Lost



Only Streptokinase (Low Dose)

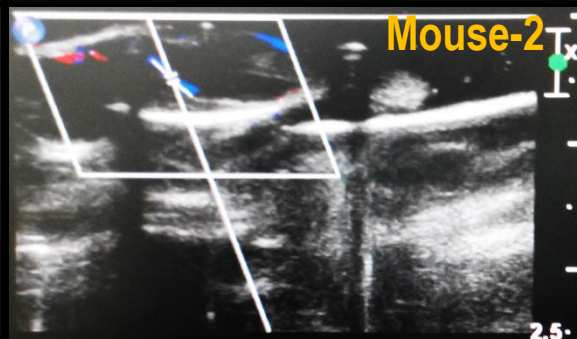
Pulse Lost



pulse wave



Pulse Lost



Streptokinase (Low Dose) + LASER

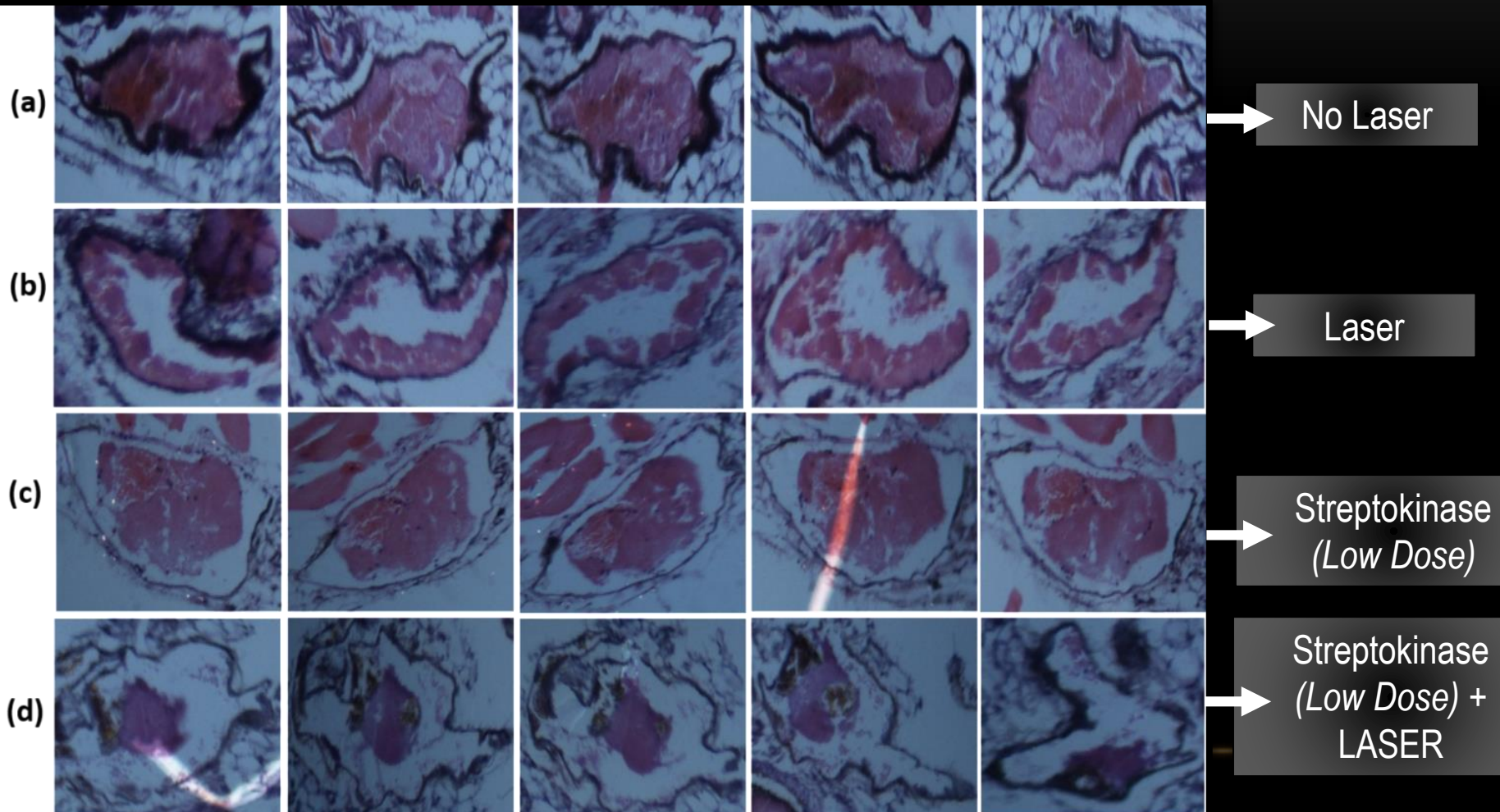


Doppler Scan before Clot Formation showing Routine Blood Flow

Doppler Scan after Clot Formation showing Occluded Blood Flow

Doppler Scan showing Restoration of Blood Flow after Laser & Low Dose Streptokinase

# Hematoxylin-Eosin Stained Transverse Sections of Mice Femoral Veins



Magnification 10x

# PHOTOTHERMAL THERAPY

- Targeted to lesion site
- Hemorrhagic complications associated with Streptokinase therapy is minimized, when low dose Streptokinase (Chemotherapy) is combined with Photothermal Therapy  
(MULTIMODAL APPROACH)

# PUBLICATION

Singh, N., Varma, A., Verma, A., Maurya, B.N. & Dash, D. (2016) *Nano Res.* 9: 2327-2337 (Relief from vascular occlusion using photothermal ablation of thrombus with a multimodal perspective) (Impact Factor: 8.893) (cited by 'Nature India')

---



# PATENT

**Patent Application No. # 3168/DEL/2014, dated 03.11.2014** on “A FIBRIN-TARGETING DEVICE WITH NIR-ACTIVE NANOMATERIALS FOR IMPROVED THROMBOLYSIS EMPLOYING PHOTOTHERMAL (PT) METHOD”

# LAB MEMBERS



# ACKNOWLEDGEMENT

- **DST Nanomission**
- **DBT**
- **ICMR**
- **Tata Innovation Fellowship**



***THANK YOU!***



**THERE'S PLENTY OF ROOM AT THE BOTTOM**  
THERE'S PLENTY OF ROOM AT THE BOTTOM

---

**Richard P Feynman (*American Physicist*)**  
**(1959 Speech)**

# Nano World

Deals with structures in the length scale of  
about **1 to 100 nm** ( $1 \text{ nm} = 10^{-9} \text{ m}$ )  
(*1 nm is roughly the width of 10 hydrogen  
atoms*)

# Examples of Nanomaterials

- Gold nanoparticles / nanorods
- Silver nanoparticles
- Carbon nanotubes (single-walled and multi-walled)
- Graphene
- Nanodiamond
- Magnetic ( $\text{Fe}_3\text{O}_4$ ) nanoparticles
- Quantum dots (Nanoscale Semiconductors), and so on.

# PART-2

## PHOTOTHERMAL ABLATION OF THROMBUS *USING GOLD NANORODS*