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Winter College on Micro and Nano Photonics for Life Sciences

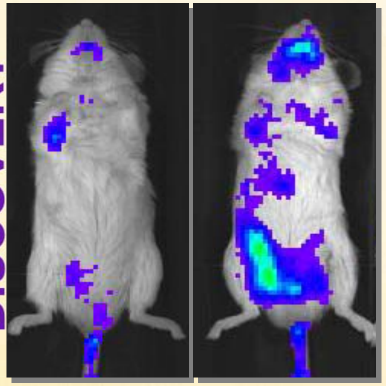
11 - 22 February 2008

Laser Trapped Raman Spectroscopy of Cellular Identification and Sorting

Dennis Matthews
NSF Center for Biophotonics
UC Davis, Sacramento
CA, U.S.A.

EDUCATION

DISCOVERY



TECHNOLOGY

Application of Ultra Short Pulse Lasers to Bioscience and Medicine – ICTP Lecture 3

Dennis Matthews, PhD

Professor, Neurological Surgery, Biomedical Engineering, Applied Science

Director, Center for Biophotonics

UCDAVIS
UNIVERSITY OF CALIFORNIA

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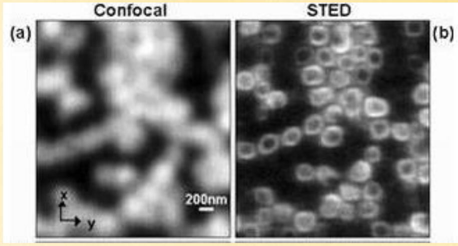
http://cbst.ucdavis.edu • dlmatthews@ucdavis.edu • (916) 734-4342

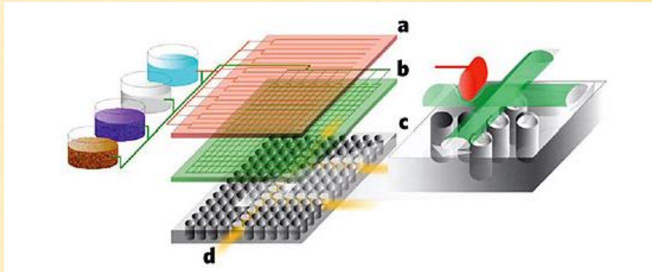
Work supported by the National Science Foundation Cooperative Agreement No. PHY-0120999

Future Directions in Biophotonics*

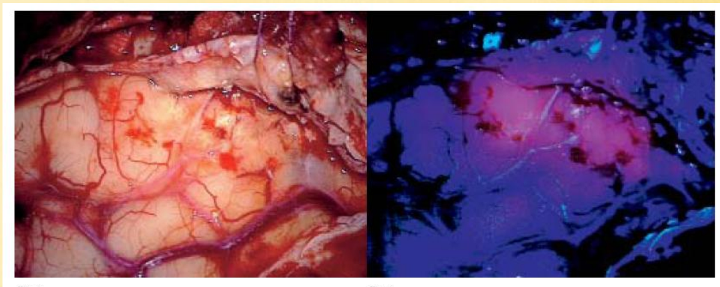
- Microscopic Imaging
  - Biomolecular imaging with X-diffraction
  - Non-linear imaging devices
    - Unlabeled viral, bacterial dynamics
    - In vitro imaging of protein complexes
  - ……………………..
- Sensors/Assays/Probes
  - POCT Devices (Optofluidic Lab on a Chip)
  - Highly targeted nano-particle probes
  - Probeless, Raman Flow Cytometry
  - Personal health monitors, metabolometers
  - High speed wide field array readers
  - …………………..
- Clinical Diagnostics/Therapy
  - Real time pharmaco-kinetics
  - Biodosimeters (radiation,viral, bacterial)
  - Response to therapy monitors
  - POCT devices for diagnoses, staging
  - Image-guided micro/nano-surgery
  - Non-invasive cancer, etc. therapy
  - Stem cell ID, tracking
  - Self-reporting *In Vivo* Nano-clinics



From Stefan Hell, Phys Rev Letts, 2005.



Developing optofluidic technology through the fusion of microfluidics and optics
Demetri Psaltis, Stephen R. Quake and Changhuei Yang
Nature **442**, 381-386(27 July 2006)



Fluorescence-guided malignant glioma resection, courtesy of Zeiss Inc.

* Caveat - I am better at attempting to create the future than predicting it!

Examples of sub ps lasers

1. **Dye lasers**: pulses as low as 10fs, wide spectral range by changing dyes. They are less used since the development of solid state lasers.

2. **Solid state lasers**: they are diode pumped, and they use

a) Transition-metal doped crystals

Ti-Sapphire lasers, with or w/o CPA (which is implemented in regenerative amplifiers) give pulses as short as 5 fs, tunability range 690-1000nm, rep rate in the kHz-100MHz;
Chromium forsterite, tunable between 1170-1340nm (difficult to achieve with other lasers), potentially good for ablating tissue because of low water/tissue absorption

b) rare-earth doped crystals

(Nd: YAG - YAG lasers - or Nd:YVO4 - Vanadate lasers)

Mode-locked high power thin disk lasers (Yb:YAG at 1030nm) deliver the highest average power (80 W) at pulse energies of several microJoules and subpicosecond (700-800fs) operation. Shorter pulses are possible with Ytterbium-doped tungstate crystals (such as Yb:KGW).

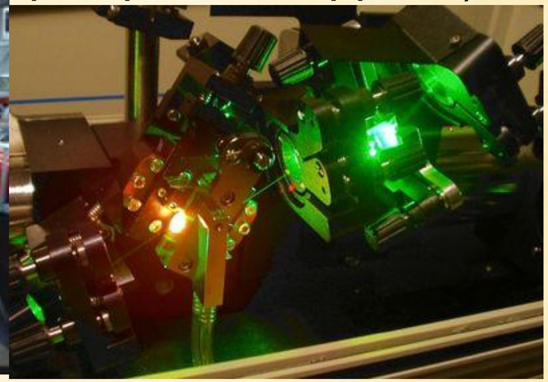
Solid state lasers, once the choice for subpicosecond, high power lasers, now have strong competition from fiber lasers.

3. **Fiber lasers**:

Mode-locked fiber lasers: the gain medium is a fiber doped with rare-earth ions (Erbium, neodinium, ytterbium, thuliom, or praseodymuim), delivering fs-ps pulses with a large gain bandwidth (enabling wide wavelength tuning range). The performance of sub-ps fiber lasers and amplifiers is limited by the strong nonlinearities of fibers and/or chromatic dispersion.

Example: fs Erbium Fiber laser at 1535nm,

Short Pulse Lasers

Research lab-sized (e.g., Nd-YAG pumped,Ti-Sapphire)

PolarOnyx

Commercial, compact fiber-based lasers (e.g., Yb doped fiber)

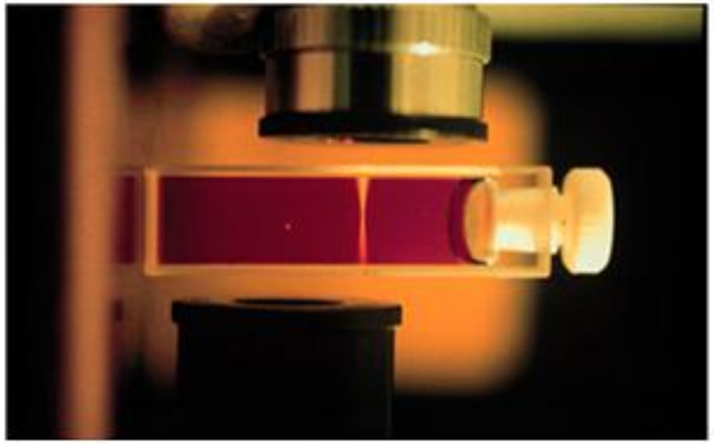
RAYDIANCE

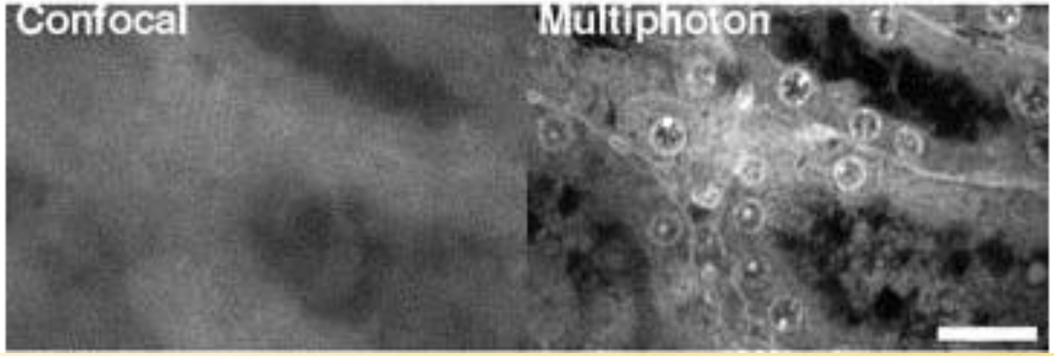
Some Imaging Applications of <ps lasers

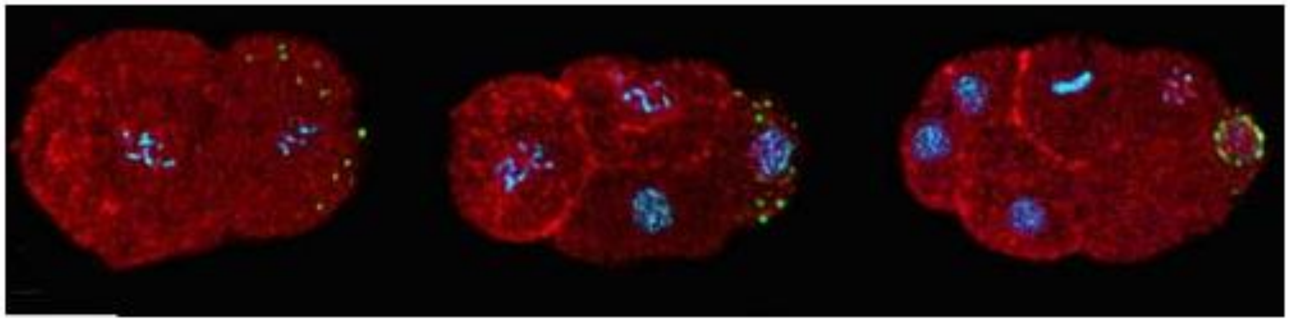
- Multiphoton Fluorescence Microscopy (laser scanning confocal microscopy)
- Fluorescence Lifetime Imaging Microscopy
- Time Correlated Single Photon Counting
- Broadband sources for Optical Coherence Tomographic Microscopy (e.g., YbDFL, 400 mW avg, BW > 30 nm, 170 fsec, ~ 1.5 micron resolution)
- THz sources
- Laser Produced X-rays (soft to Bremmstralung)
- X-ray FEL (coherent, ultrashort pulse, diffraction limited) Diffraction Imaging
- Multiphoton Harmonic Generation
  - Tunable coherent light source to XUV, soft x-ray regime

Imaging

- Multiphoton Fluorescence Microscopy (laser scanning confocal microscopy)







Combination 2-photon (red and green) and 3-photon (blue) image of C.elegans embryo

Imaging

- Fluorescence Lifetime Imaging Microscopy

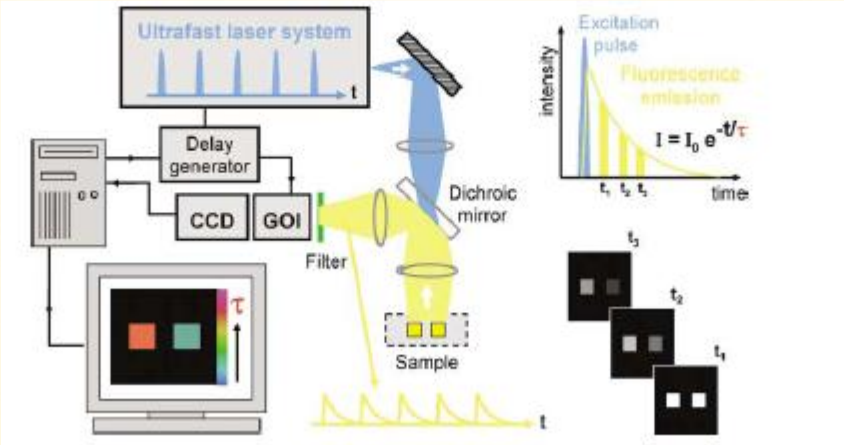


Fig. 1 Schematic of wide-field time-domain FLIM.

Paul French, Imperial College

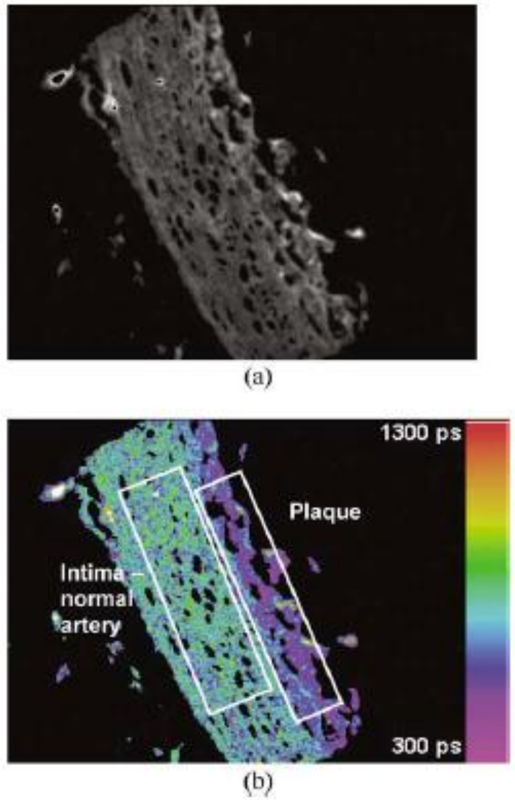
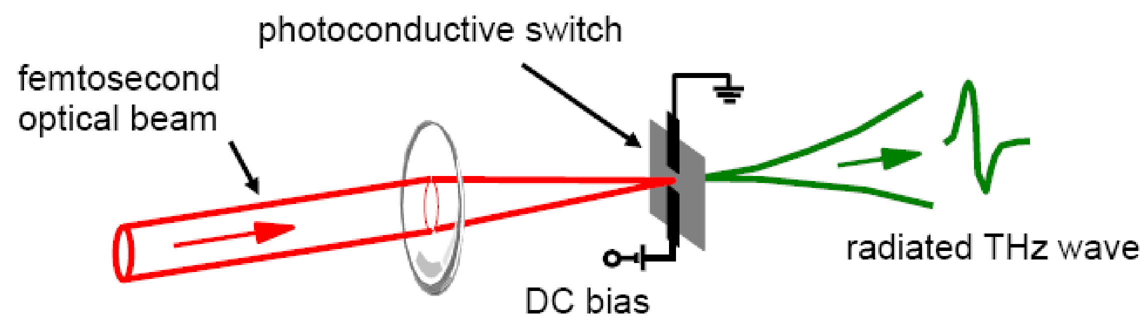


Fig. 7 (a) Wide-field fluorescence image and (b) wide-field FLIM image of unstained section of rabbit artery with atherosclerotic plaque.

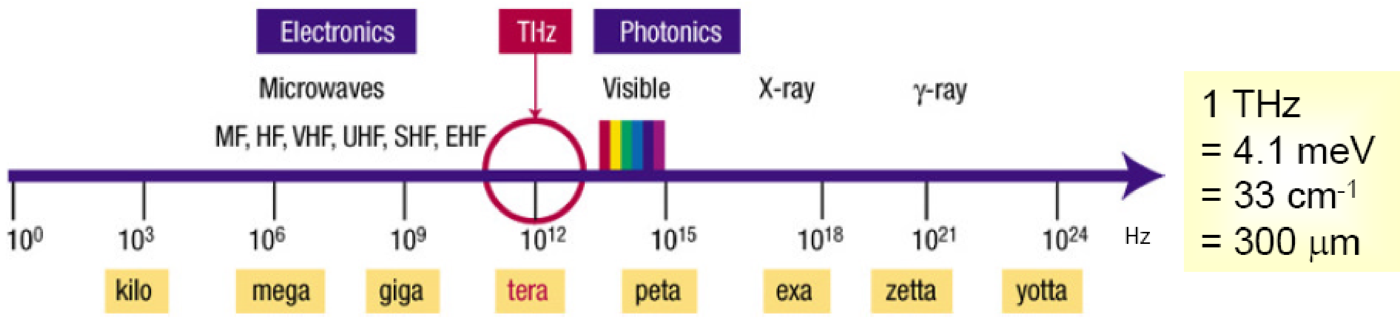
Imaging

- THz sources



The Terahertz (THz) Gap

"The most scientifically rich, yet underutilized region of the EM spectrum" –Tom Crowe

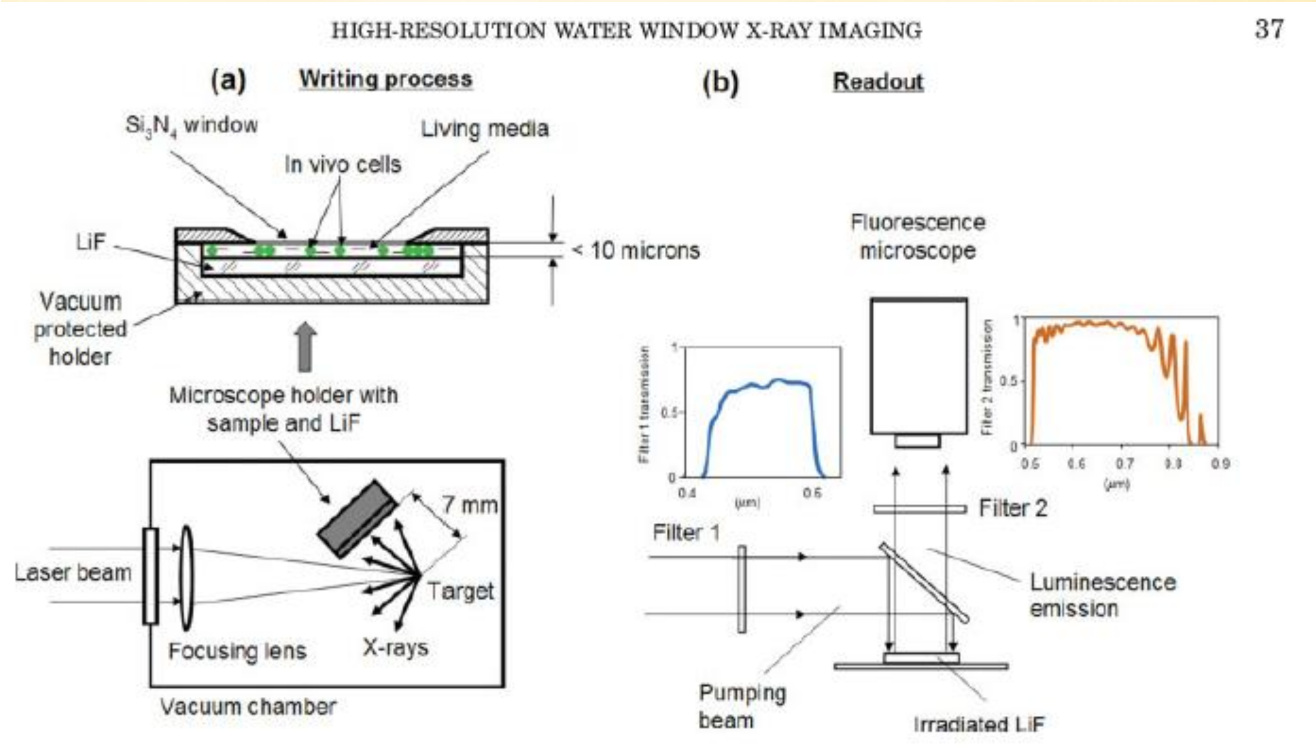


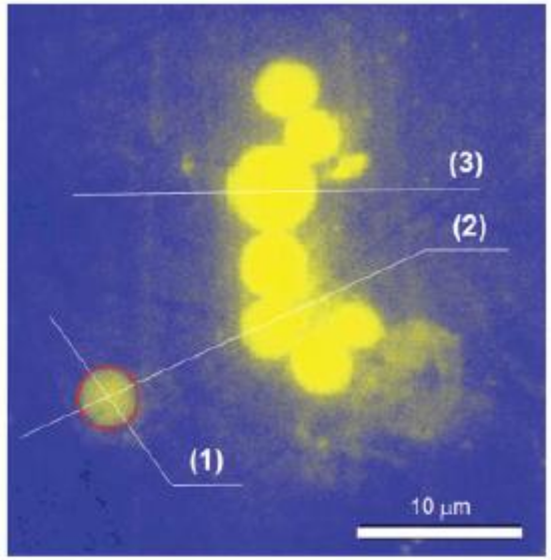
THz Science: collective excitations, protein motions & dynamics, superconductor gaps, magnetic resonances, terabit wireless, medical imaging, security screening, detecting explosives & bio agents …

Michael C. Martin, Lawrence Berkeley National Lab

Imaging

- Laser Produced Plasma X-ray Source for Live Cell Imaging



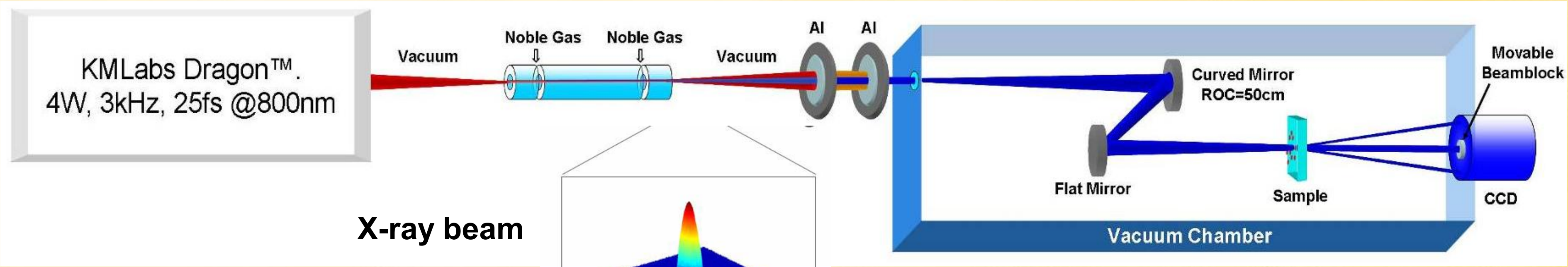


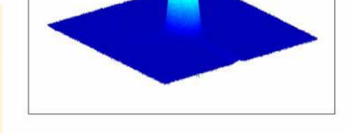
In vivo *Clorella* cells

Bonfigli et al., Microscopy Research and Technique 71, 35 (2008).

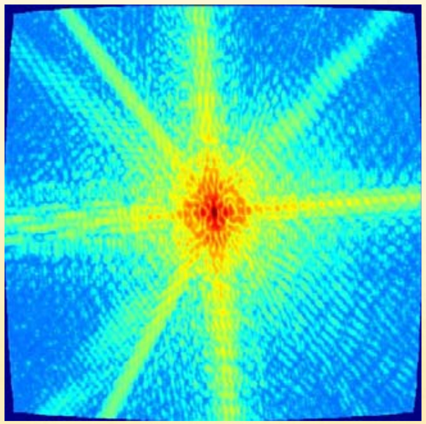
Multiphoton Harmonic Generation

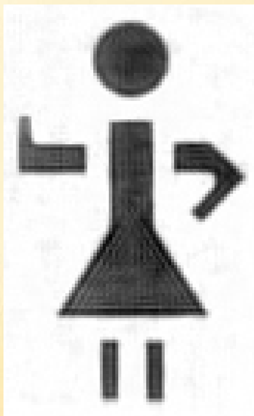
Tunable coherent light source from fundamental to 25th or more harmonic – Table top coherent x-ray source for diffraction imaging

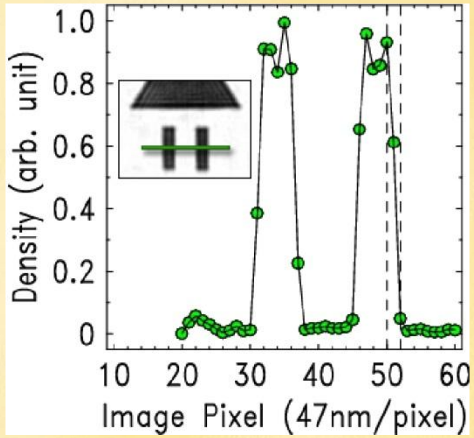




Anne Sakdinawat, Dave Attwood (**Berkeley**)
Mario Marconi, Jorge Rocca, Carmen Menoni (**CSU**)
Changyong Song, John Miao (**UCLA**)
Richard Sandberg, Daisy Raymondson, MM, HK (**JILA**)







1 μm

sample (SEM image)   diffraction pattern   reconstruction

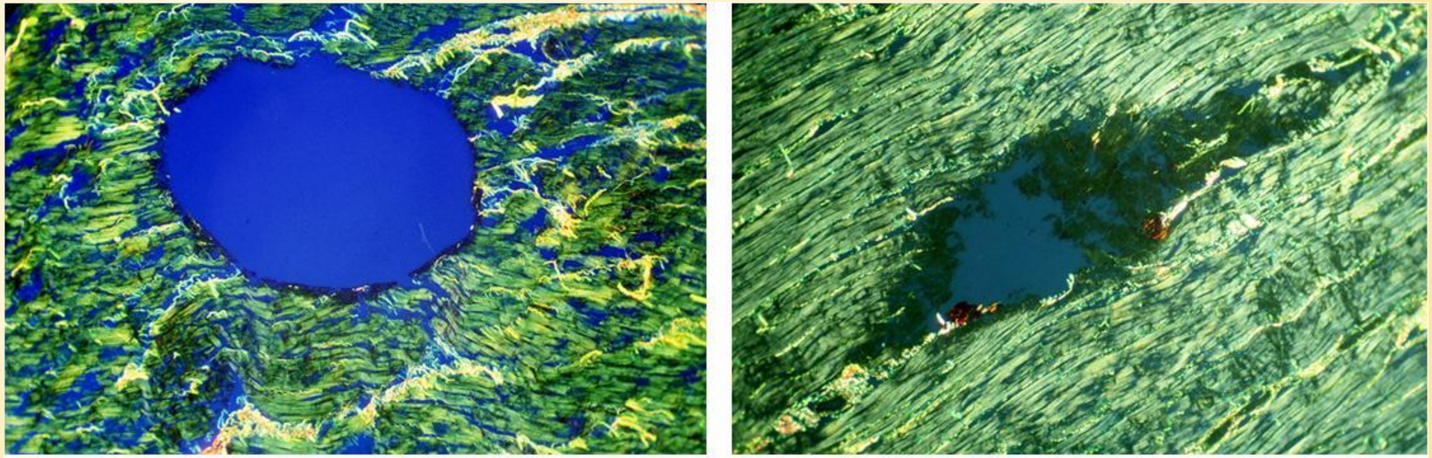
Phys. Rev. Lett. **99**, 098103 (2007); PNAS **105**, 24 (2007); Nature, News & Views **449**, 553 (2007); Nature Photonics (Feb. 2008)

Some Categories of Medical Application of Short Pulse Lasers

- Precision Cutting/Drilling
- Laser Generation of X-rays, Charged Particles for Radiation Therapy

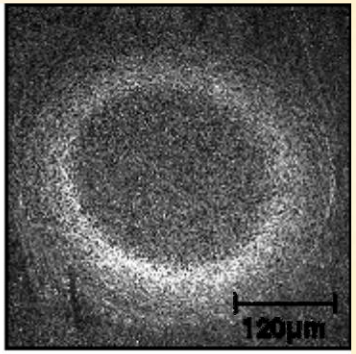
Medical Therapy Application of Short Pulse Lasers

- Precision Surgery
  - concise control of damage using pure photoablation
    - ~ depth of cut: 1μ/pulse
    - tissue independent
    - no lateral heat transport

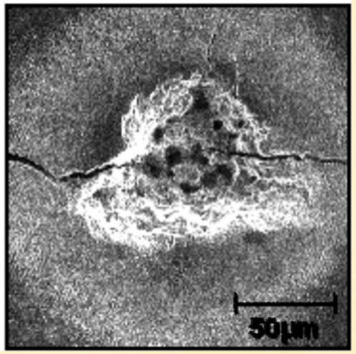


The pictures show phase contrast images of heart tissue drilled with short and long pulse lasers.

Ultrashort pulse laser tissue excision



$\tau_L \sim 0.3$ ps
$E_L \sim 3$ J/cm^2



$\tau_L \sim 1000$ ps
$E_L \sim 35$ J/cm^2

Ultrashort pulse lasers minimize collateral damage making them valuable for dentistry and microsurgery

Existing and potential fsec laser surgery markets

TABLE 1 Potential Surgeries using USPLs

Precision ablation; minimal tissue removal
- Corneal sculpting (eye: refractive correction)
- Sub-pial resection; neuron disruption (brain: seizure control)
- Stapedectomy (ear: hearing restoration)

Precision ablation; modest tissue removal
- Caries removal (teeth)
- Neurological surgeries
  - (i.e., bone resection near nerve, spine: pain reduction)

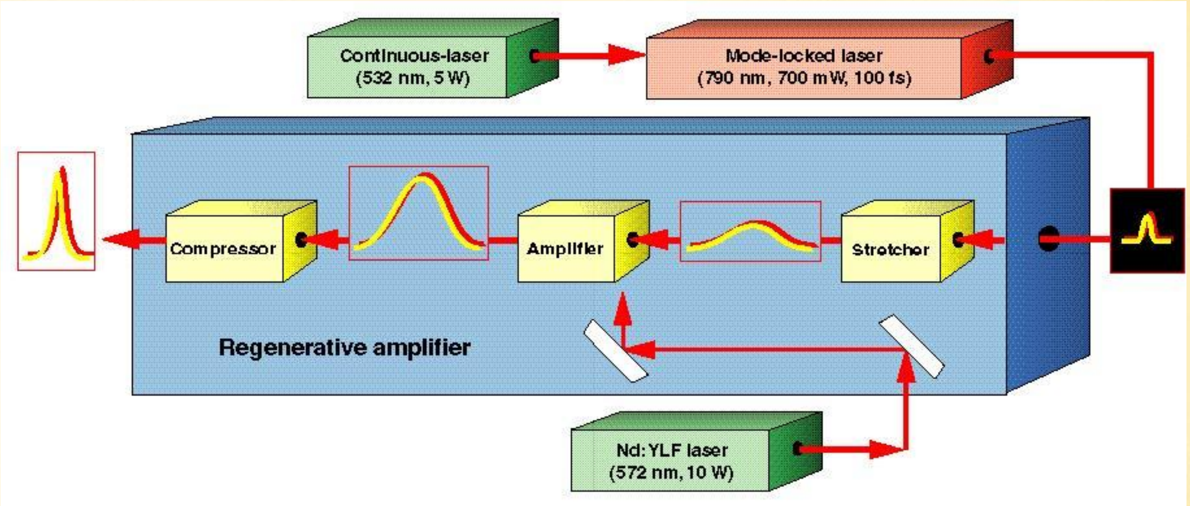
Non-precision ablation
- Diskectomy (spine: stabilization, pain reduction)
- Tumor resection
- Orthopedic surgery (i.e, knee; cartilage resection)
- Debulking in general surgery

Table 2 Patient Populations for Potential USPLs Surgeries

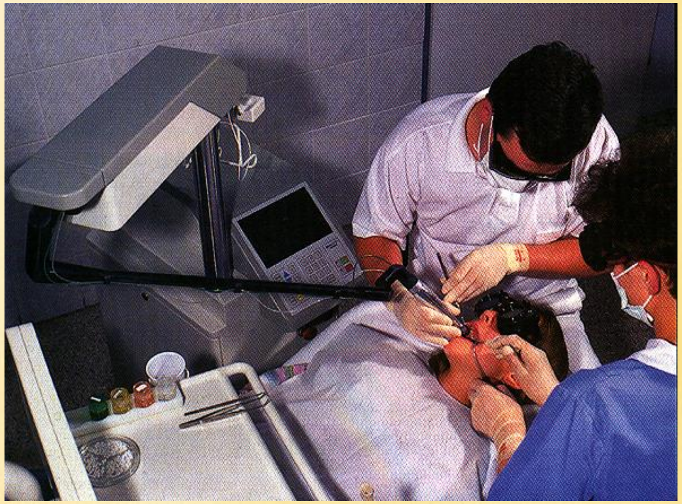
Procedure	US Annual Cases (1996)
Head, neck and skull surgeries	212, 000
Facial bones and joints	102, 000
Spinal Cord and spinal canal structures	642, 000
Other cranial nerves	69, 000
Disk surgeries*, Laminectomies*	317, 000
Eye surgeries*	283, 000
Ear surgeries	68, 000
Epilepsy surgery*	500
Skin resurfacing*	880, 000

Ultra Short Pulse Laser Therapy

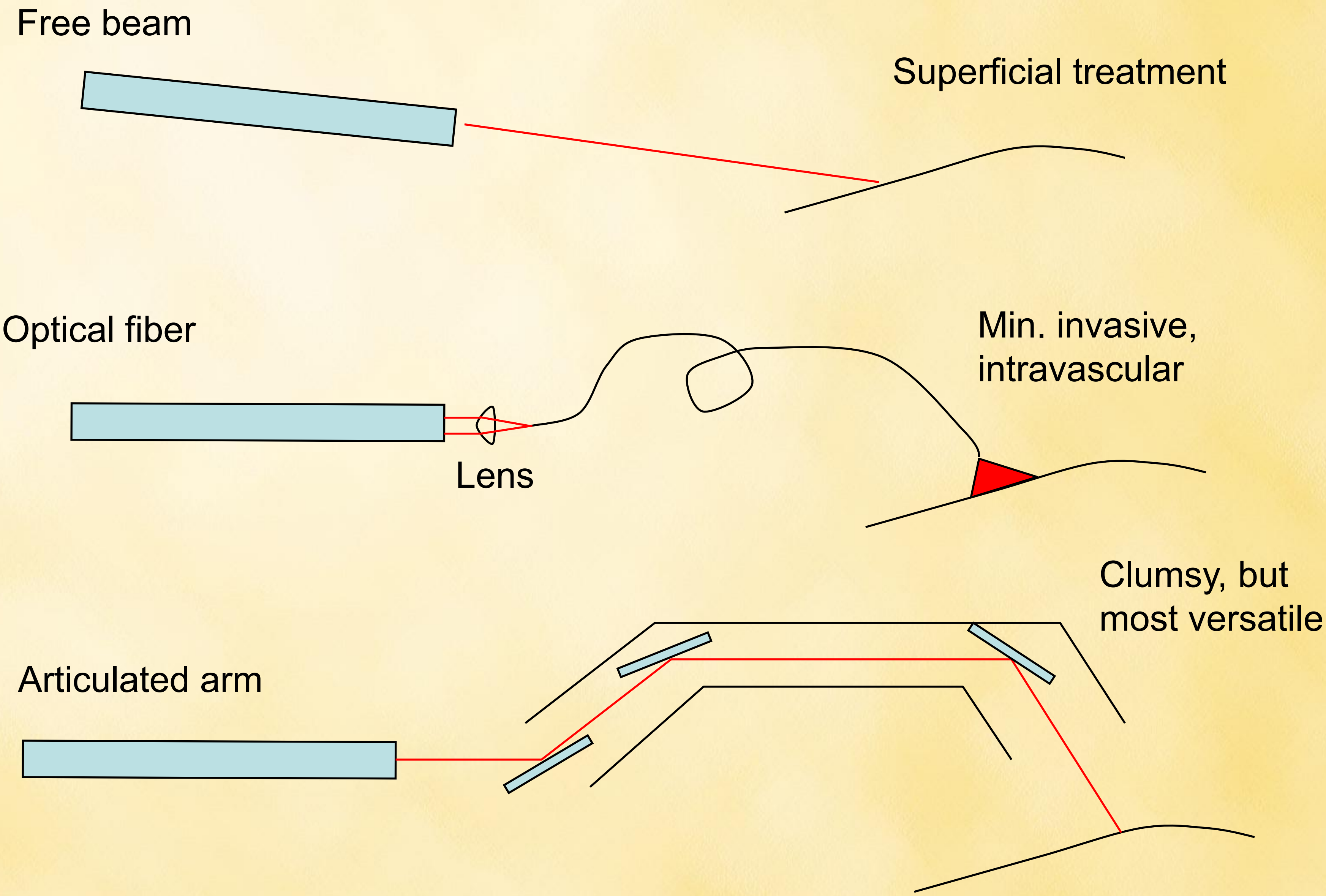
150 fsec
1 mj/pulse
1 kHz



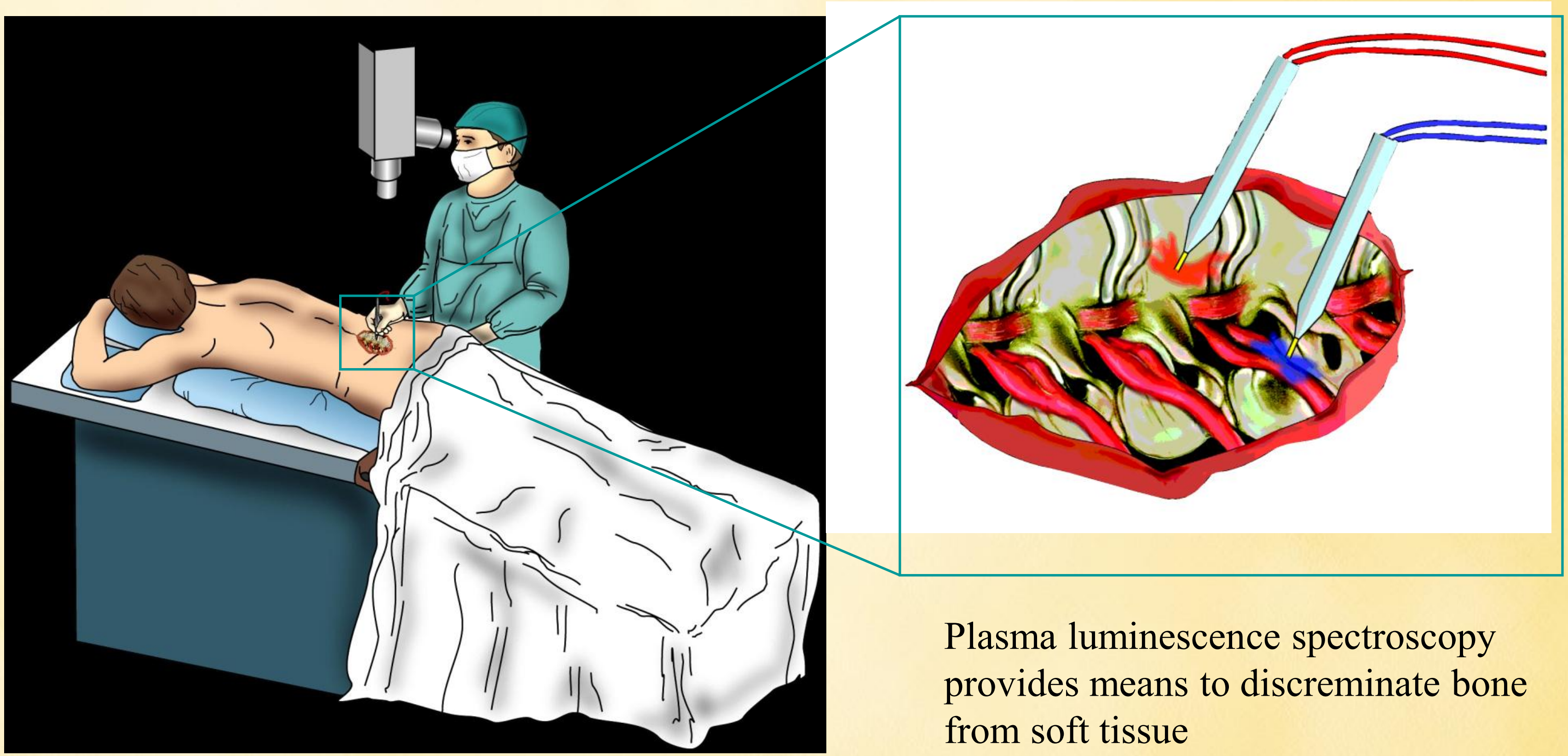
Practitioners want a high rep rate, sensor-controlled, compact system for use in dentistry and microsurgery



SPL Delivery systems

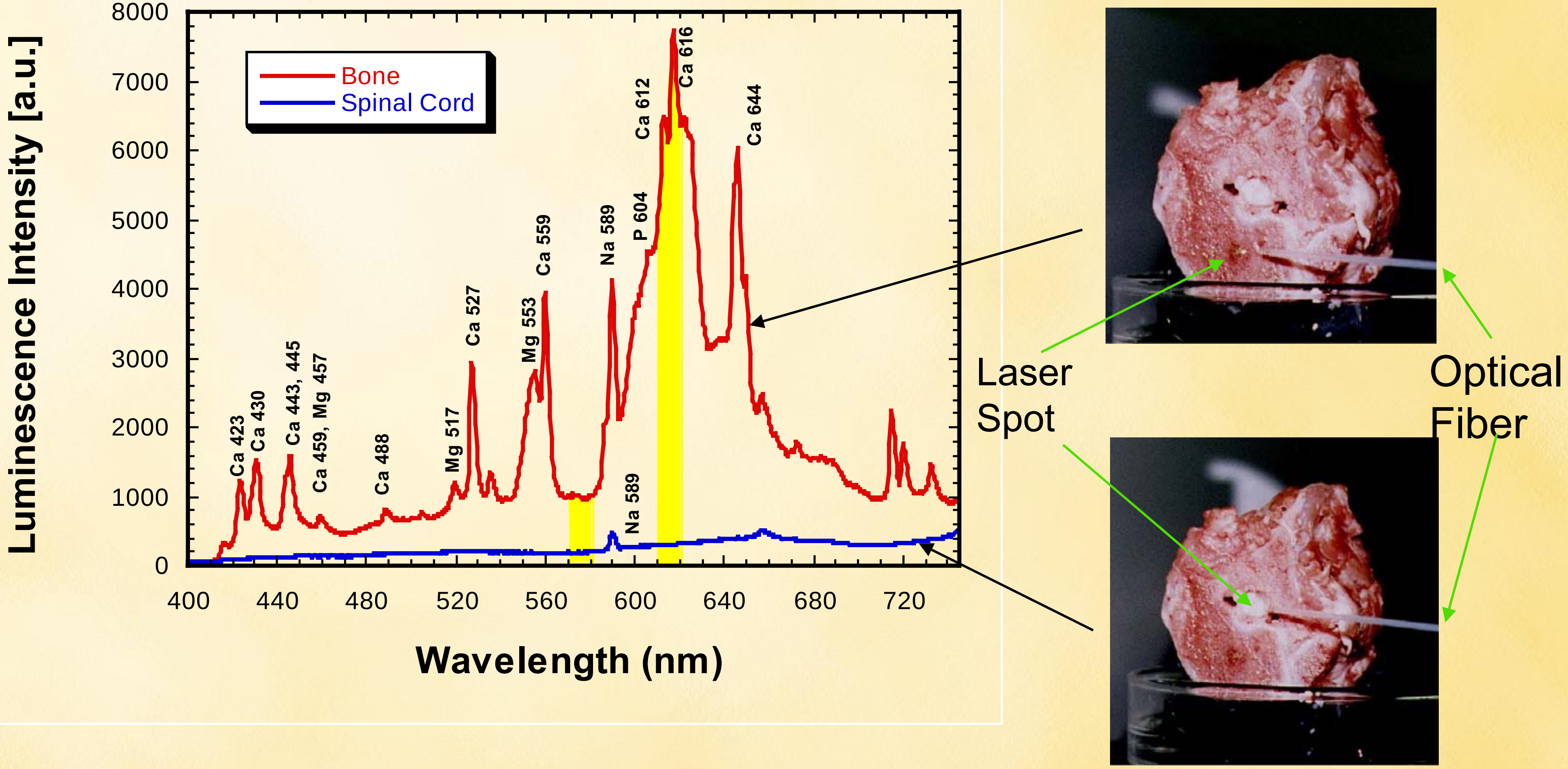


Spectroscopic sensor feedback control facilitates delicate surgery



Plasma luminescence spectroscopy provides means to discriminate bone from soft tissue

Laser Spinal Surgery



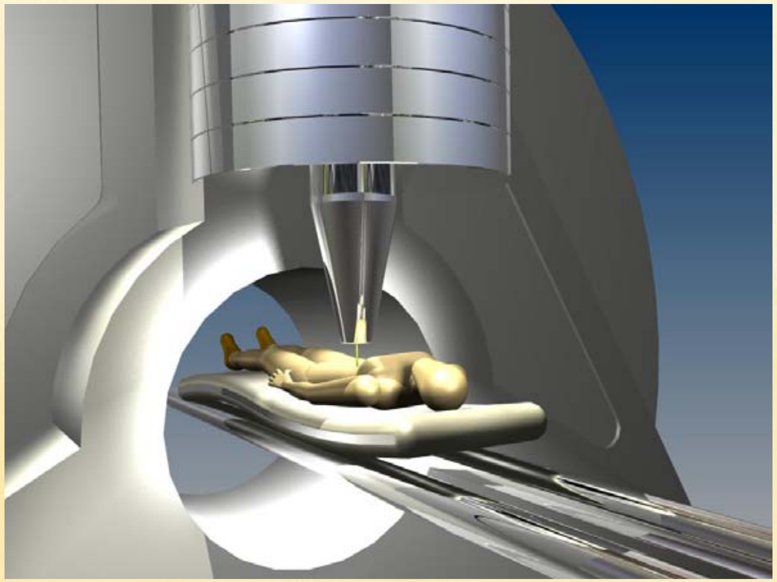
Feedback controlled ultrashort pulse laser ablation

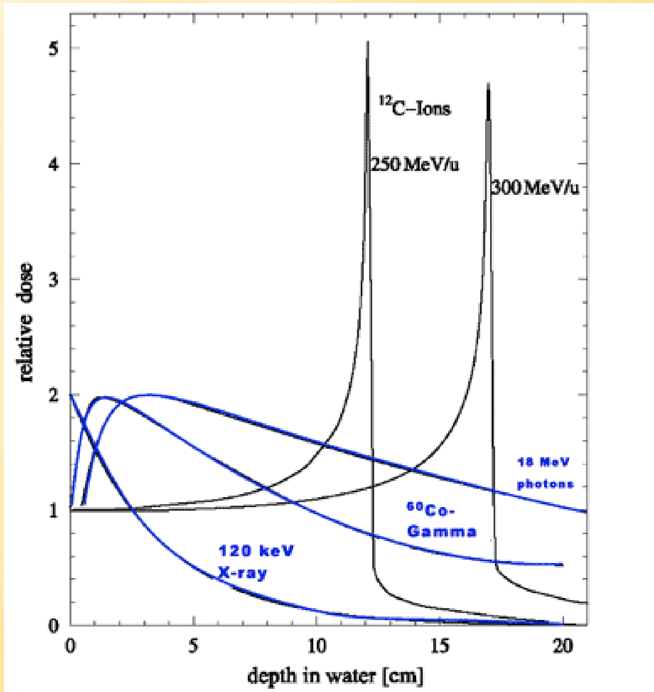
Some Categories of Medical Application of Short Pulse Lasers

- Precision Cutting/Drilling

Device Market: ~2B/yr in US

- Laser Generation of High Energy X-rays, Charged Particles for Radiation Therapy





The concept of laser particle accelerator

K W D Ledingham, W Galster and R Sauerbrey

The British Journal of Radiology, November 2007

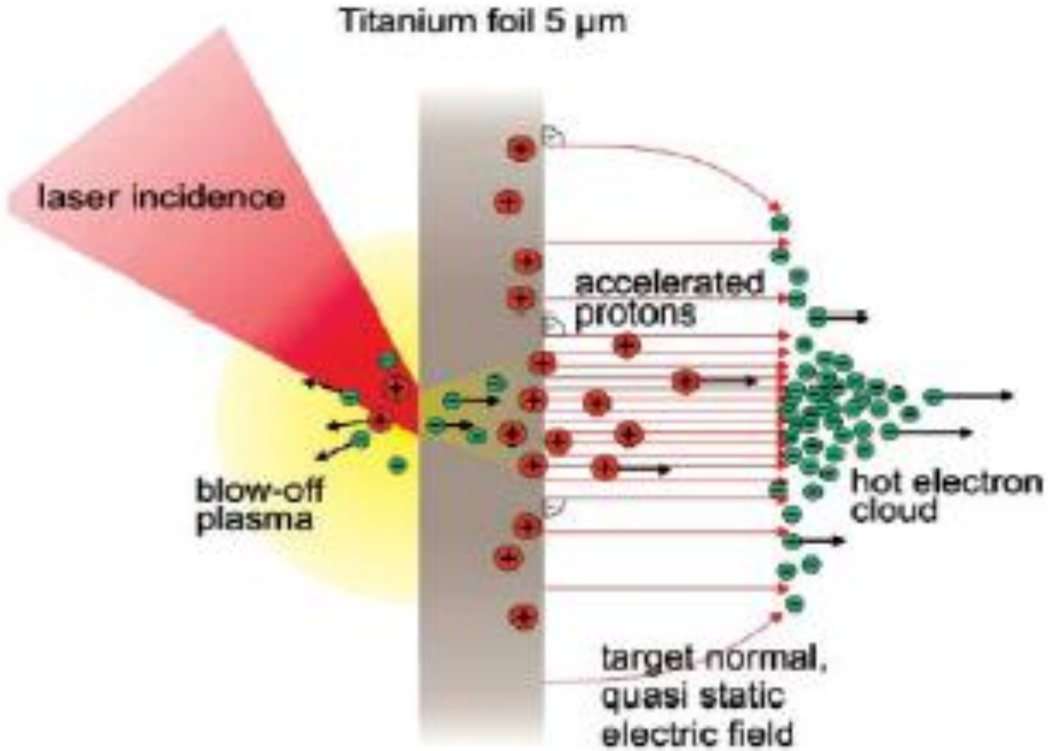


Figure 1. High-intensity laser formation of protons.

Proton particle generation with lasers

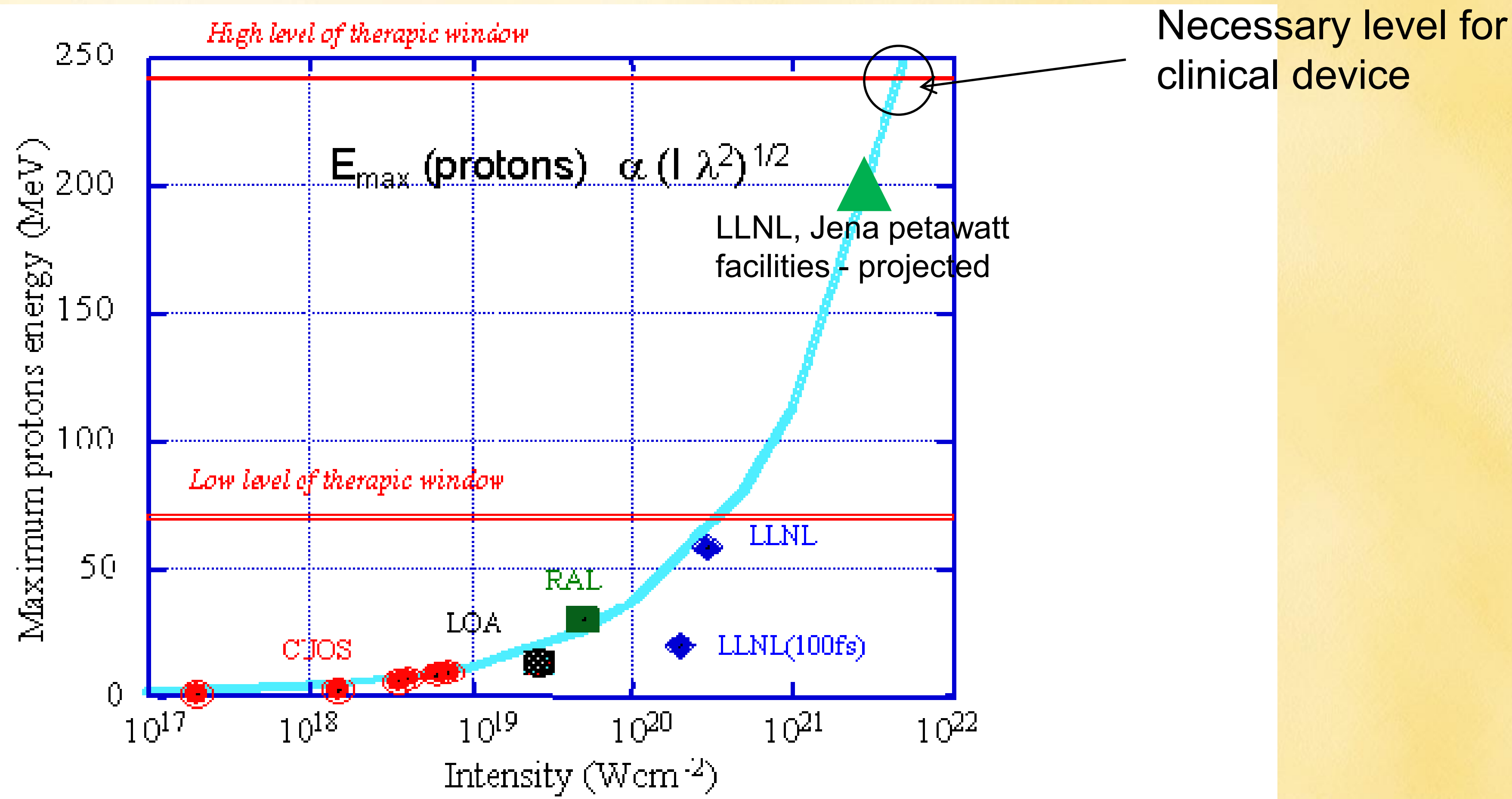


Fig. 4: Measured maximum proton energy versus the laser intensity as achieved over different facilities worldwide. From the blue curve $(\lambda I^2)^{1/2}$ scaling law λ that is the laser wavelength and I its intensity-, it appears that lasers able to produce intensity near 10^{21} Wcm^{-2} at the focal spot will enter the medical region.

Issues to address with Short Pulse Lasers

- Cost (Dentists: $30-50k, Surgeons: $70-100k, max)
- Size – surgeons, dentists, dermatologists, etc. need small
  - ~ desktop computer (2’x1’x1’)
- Complexity
  - turnkey operation is imperative
  - fiber optic delivery highly desirable
  - Image- or sensor-guided critical for precision applics
- Energy/pulse (10 μJ to 1 mJ)
- Avg. Power
  - Depends on application (1-1000W)

Take-aways from this talk

- Sub psec lasers (SPL) are ubiquotuous for bioimaging applics like multiphoton fluorescence imaging
- SPL is making incredible inroads into other imaging applications like THz, Table Top X-ray, XFEL
- Laser Surgery and Drilling is a critical need that can be met by SPL
- SPL surgical devices must be cheaper, smaller and embody f/o delivery and sensor guidance
- Laser particle therapy is becoming a possibility, but cost/size of facilities must be < $10M, fit in conventional rad onc treatment facility

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