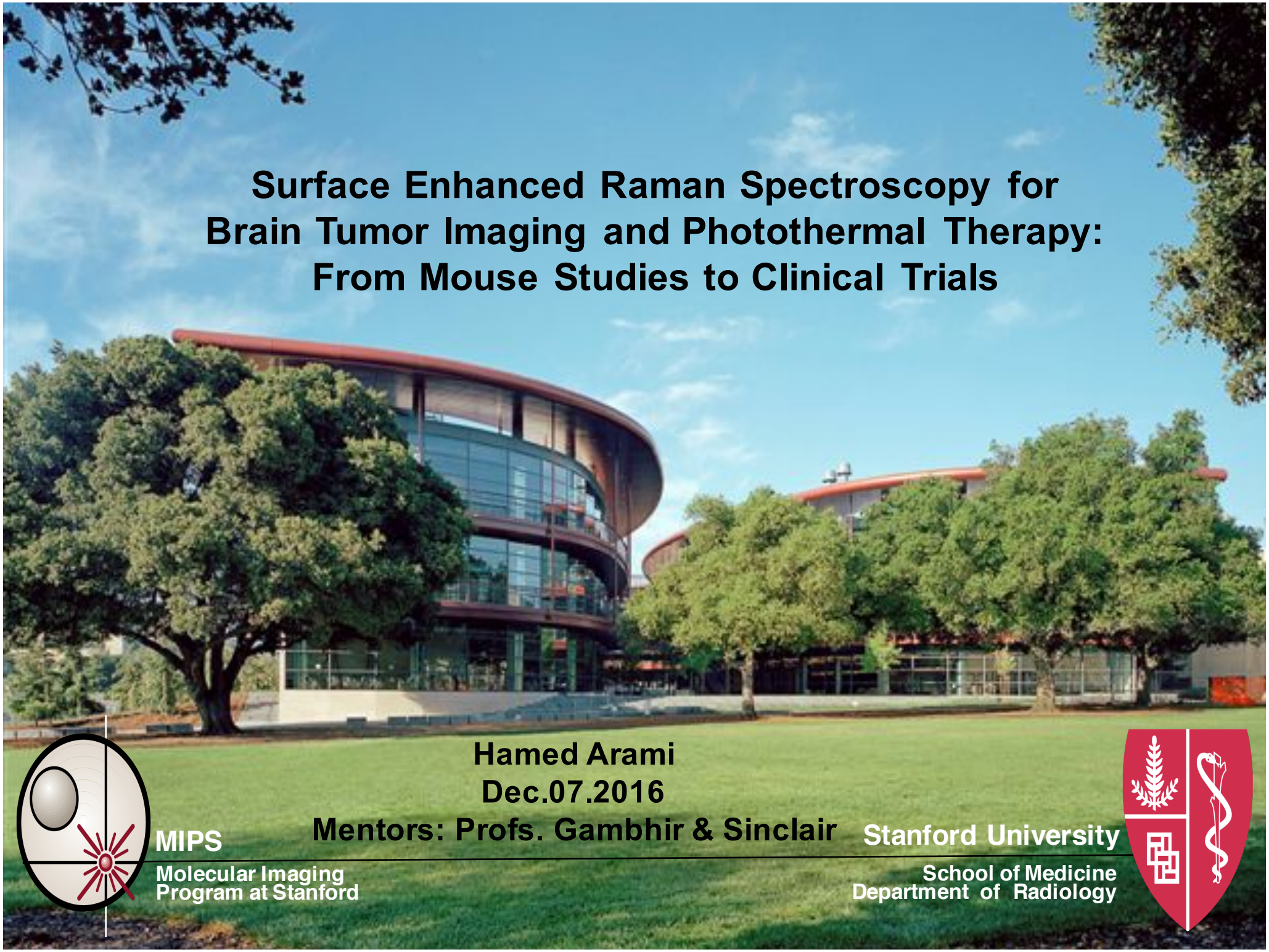
Surface Enhanced Raman Spectroscopy for Brain Tumor Imaging and Photothermal Therapy: From Mouse Studies to Clinical Trials



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Dec.07.2016

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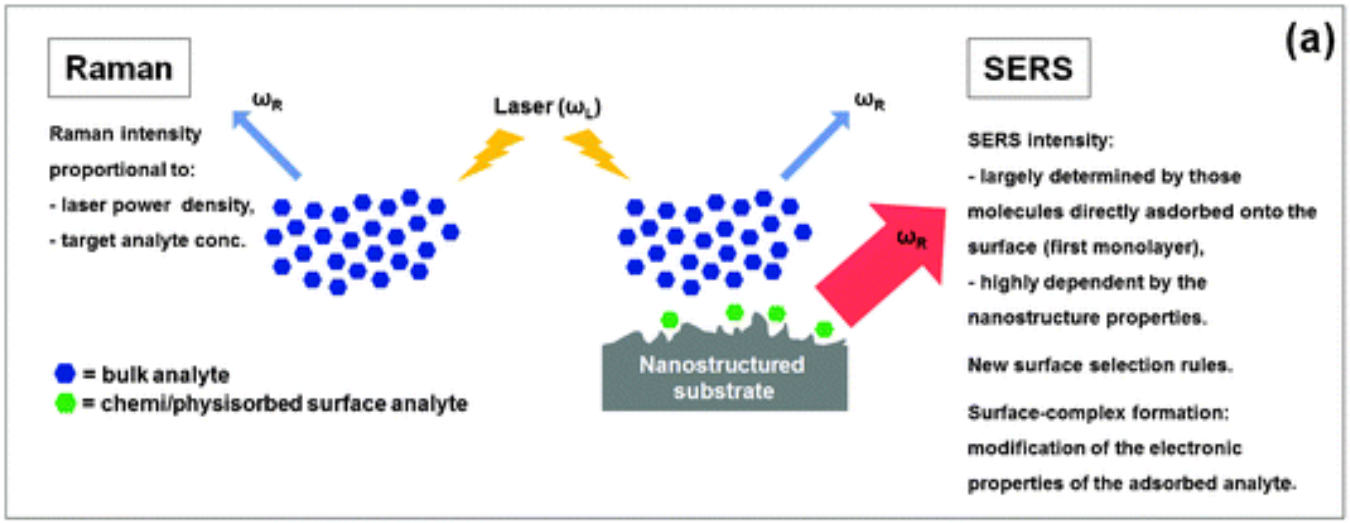
MIPS
Molecular Imaging Program at Stanford

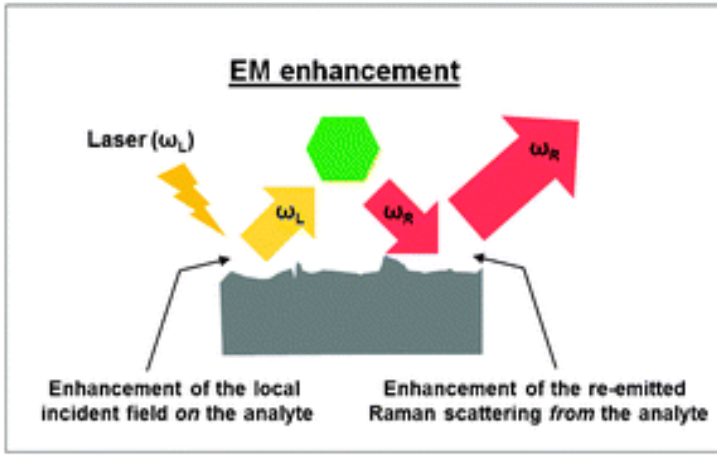
Stanford University
School of Medicine
Department of Radiology

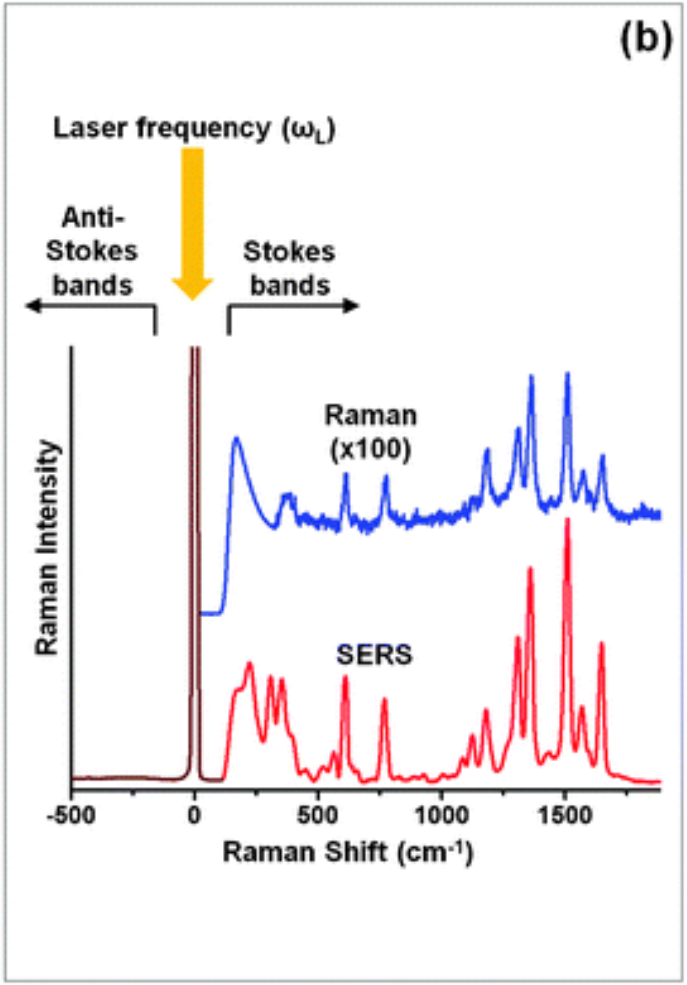
Outline:

- **Introduction**
  - Surface Enhanced Raman Spectroscopy (advantages and challenges)
- **Experiments and Results**
  - A companion clinical study to evaluate SERS technology for GBM imaging
  - SERS for intraoperative diagnosis and therapy of GBM
  - Using immune cells for delivery of SERS nanoparticles to GBM
- **Conclusions & Future Plans**

Surface Enhanced Raman Spectroscopy (SERS):

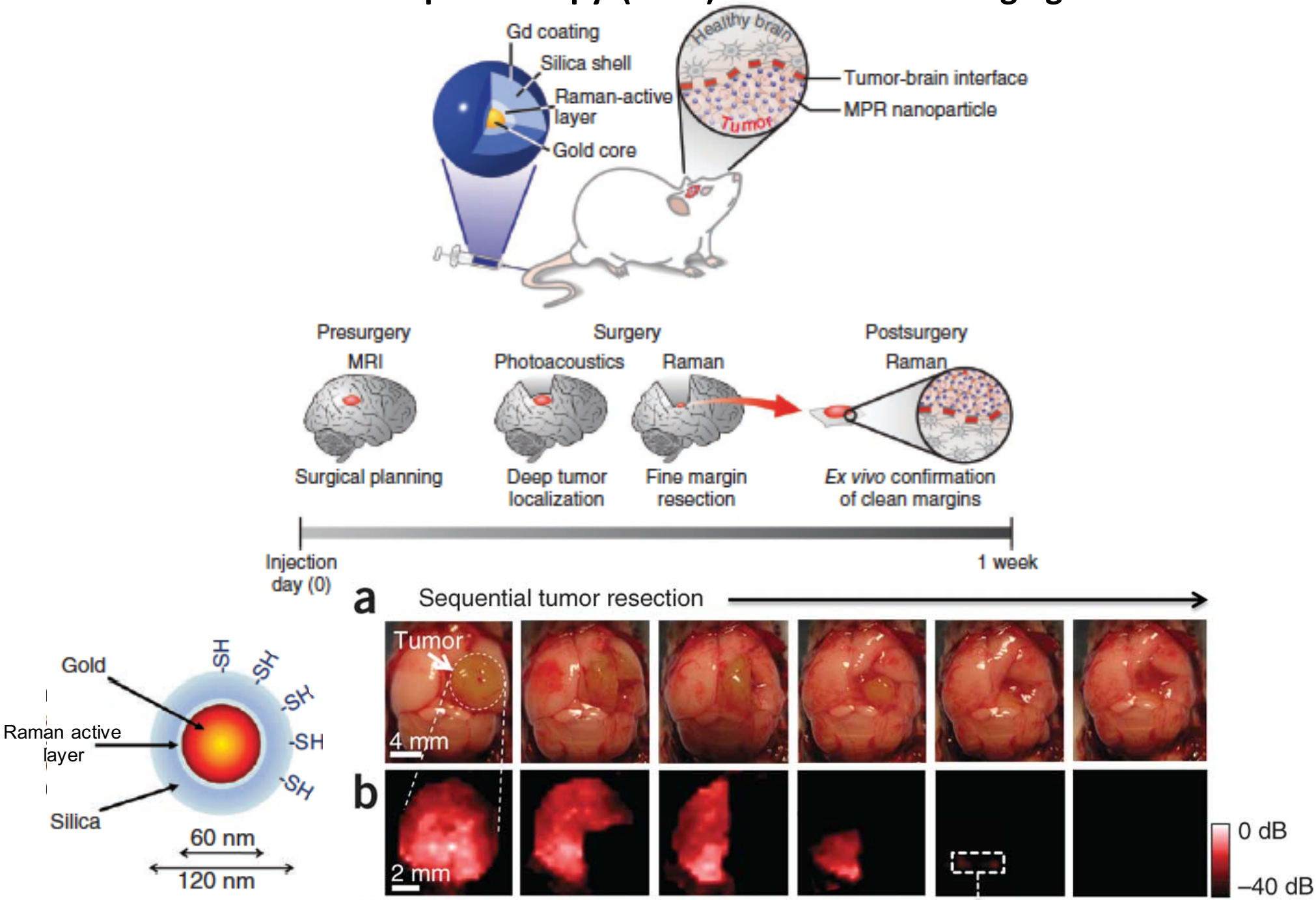






L. Guerrini and D. Graham, Chemical Society Reviews **41** (2012) 7085.

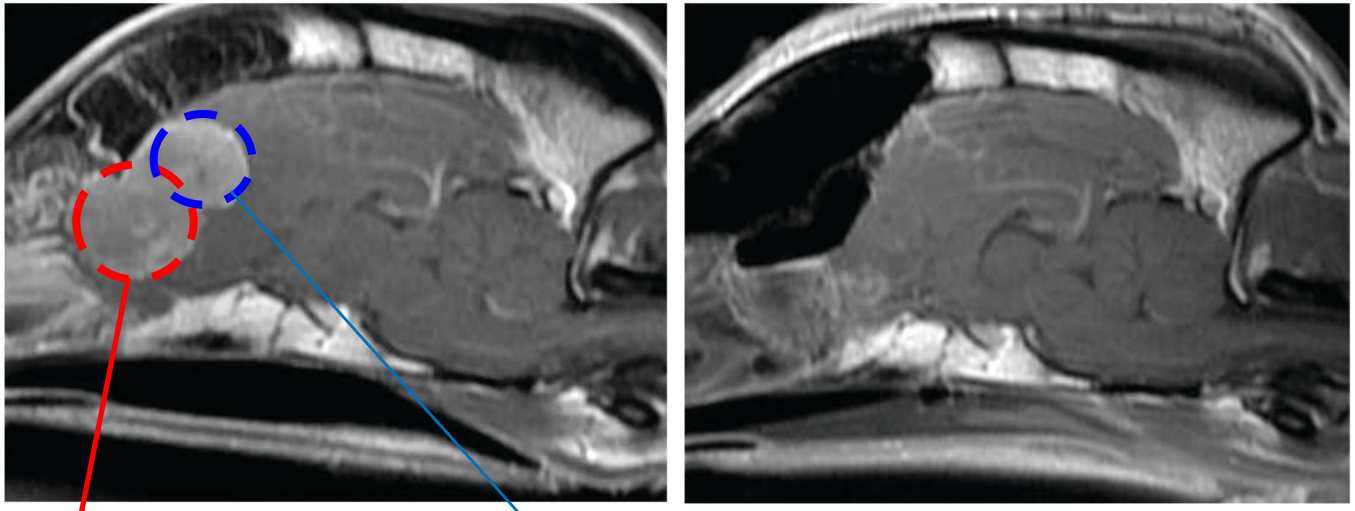
Surface Enhanced Raman Spectroscopy (SERS) in Biomedical Imaging:



Kircher et al. Nature Medicine **18** (2012) 829.

Dog Frontal Lobe Meningioma post-contrast T1 MRI

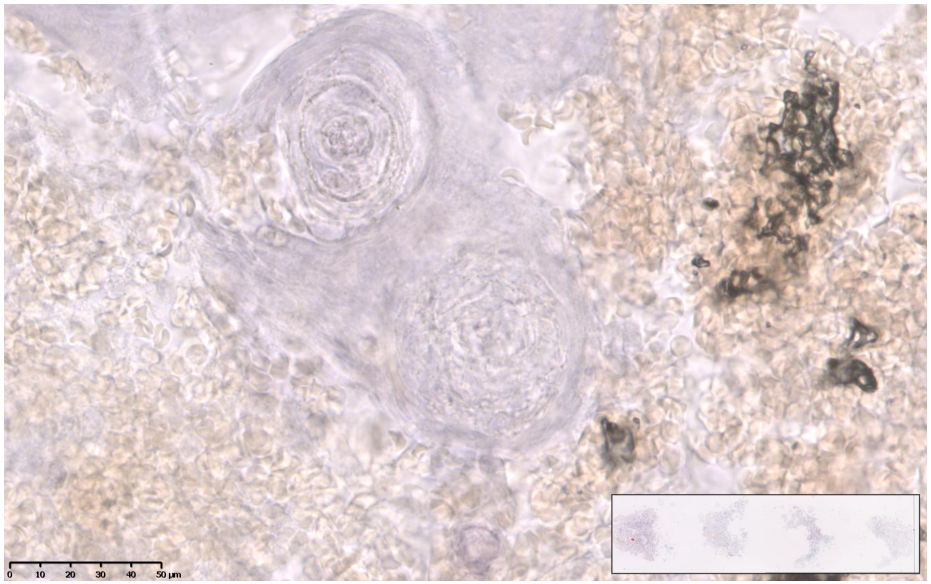
Pre-Operative **Post-Operative**



Anterior (ventral) tissue

Posterior (dorsal) tissue

Histology of the tumor tissue (H&E)



Whorls and Psammoma bodies (center) and dura mater (left, right, bottom)

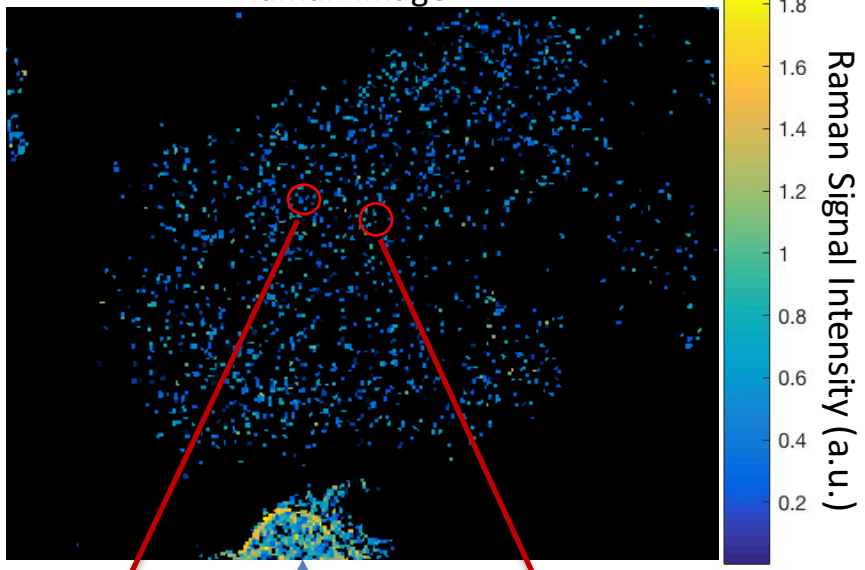
Raman image of the posterior (dorsal) tissue section (thickness : 100µm):

Posterior (dorsal) tumor tissue

Photograph of the tumor tissue slice

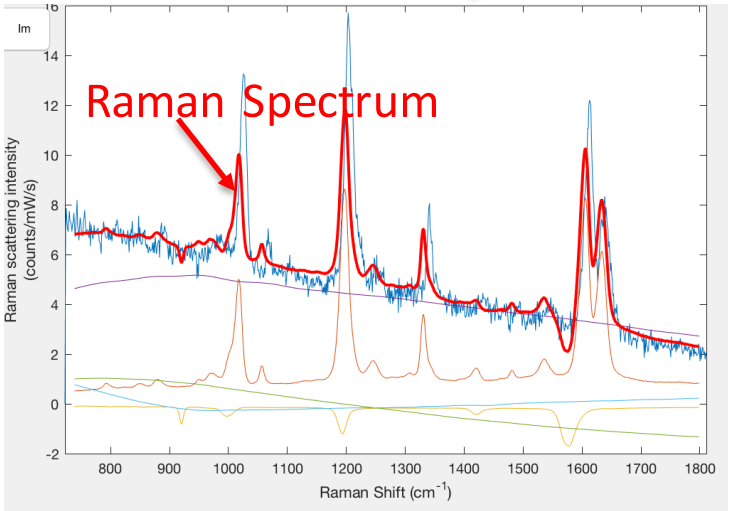
Fiducial

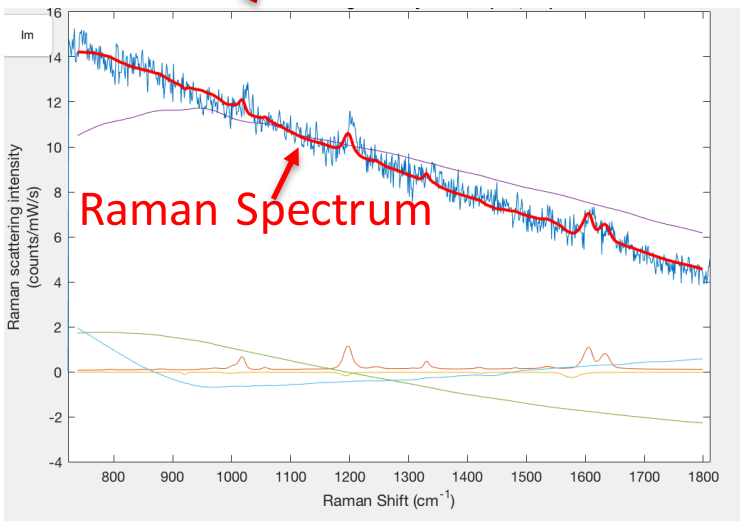
Raman image



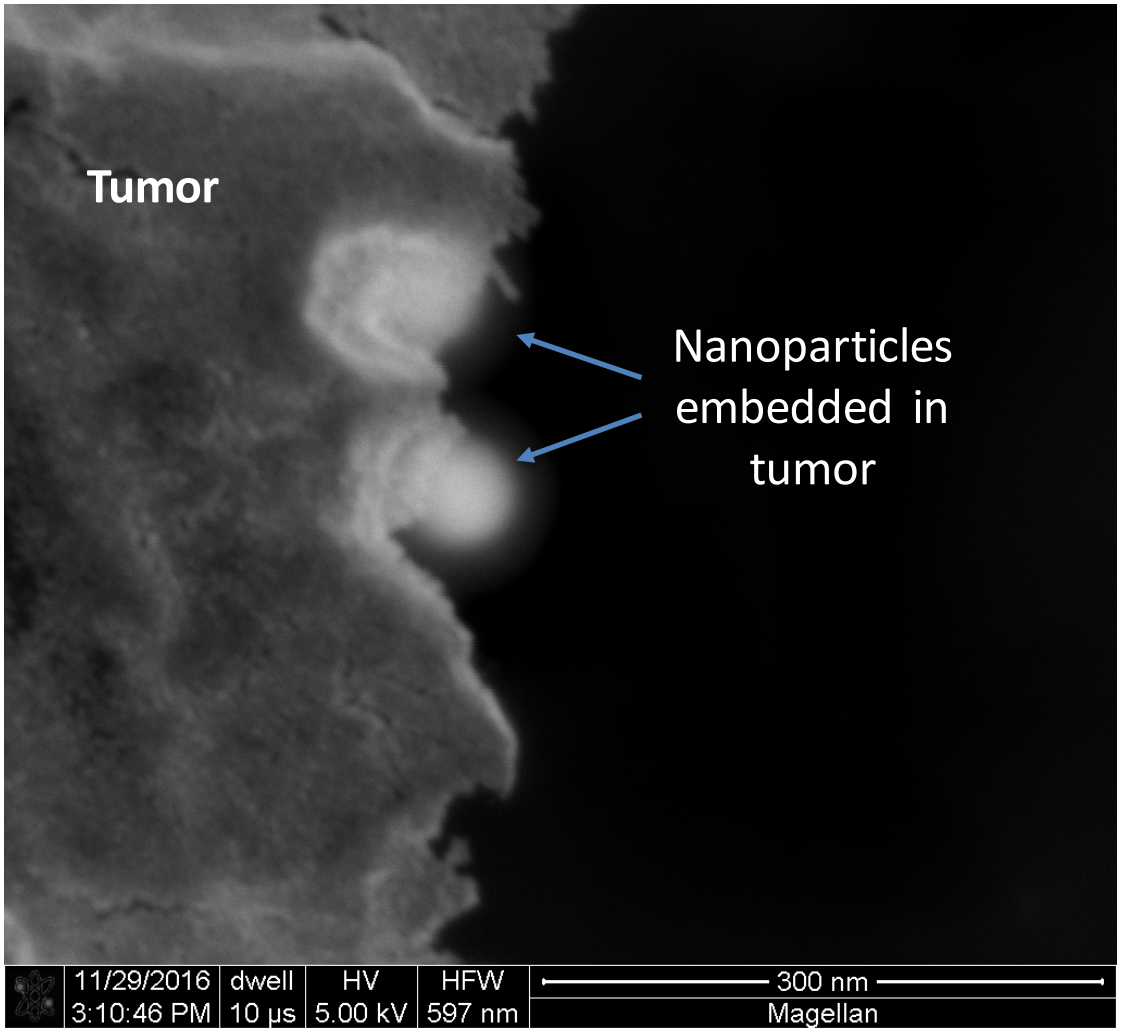
Fiducial

Resolution (step size)= 50 µm
Exposure time= 1s





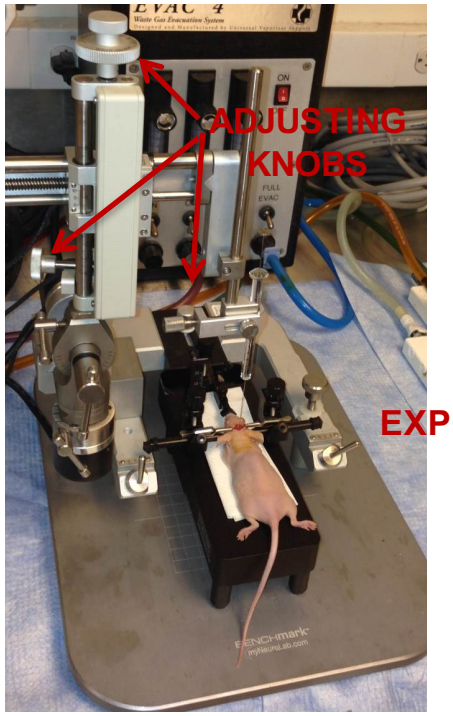
SEM at a tissue section, showing nanoparticles embedded in tumor tissue:

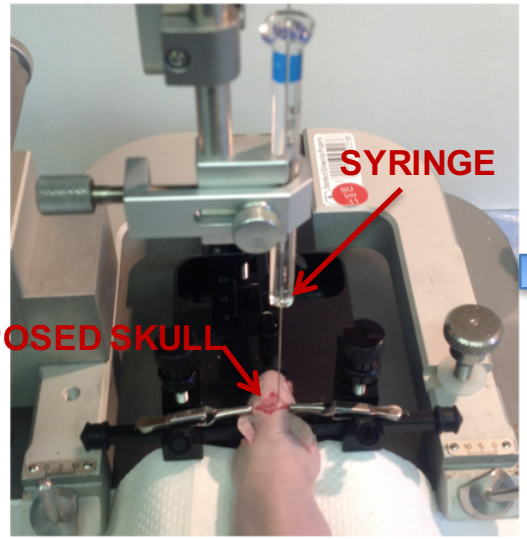


Investigation of intratumoral diffusion pattern and kinetics of Raman nanoparticles:

- No BBB challenge
- Administration of a much lower dose of the nanoparticles to tumor compared to intravenous approach
- Nanoparticles will be removed during the tumor resection procedure
- No nanoparticles in liver and spleen
- Challenging for highly diffusive types of GBM
- Challenging for deep brain tumors which are not accessible

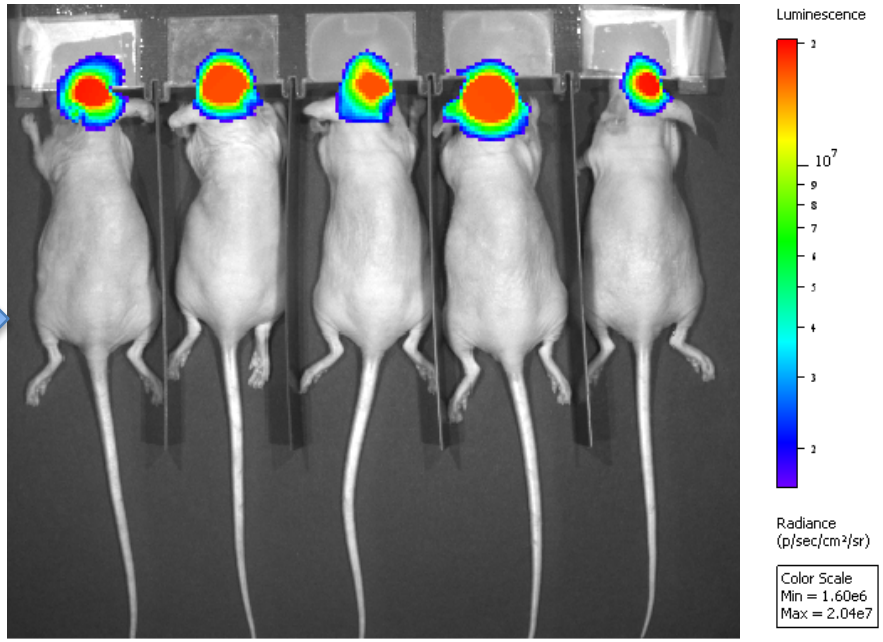
Investigation of intratumoral diffusion pattern and kinetics of Raman nanoparticles:



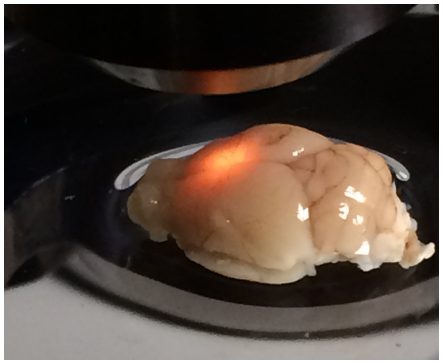


Stereotactic injections of the U87-GFP-Luc cells

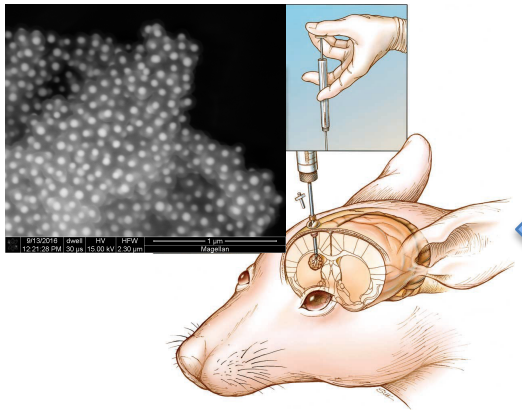
Implant – U87_GFP/fLuc



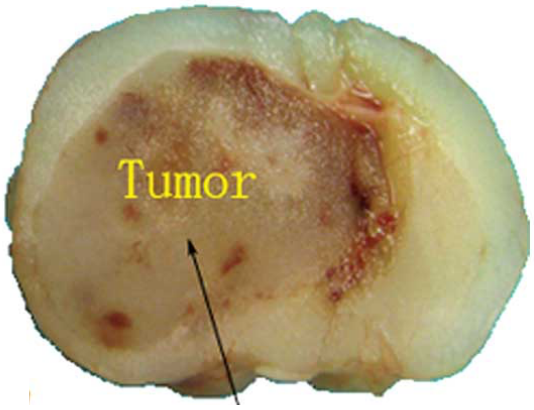
Bioluminescent images of the mice



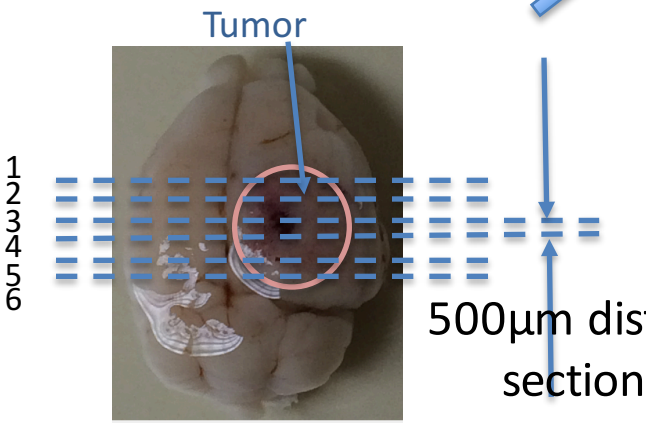
Excising the brains for analysis



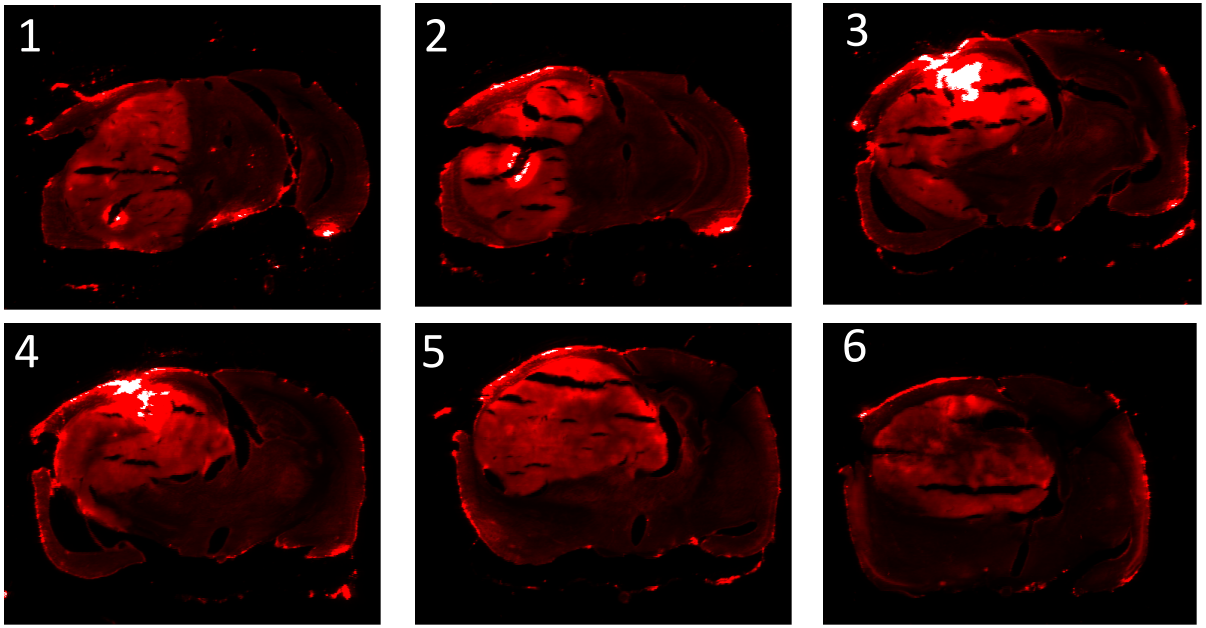
Intracranial injection of the nanoparticles



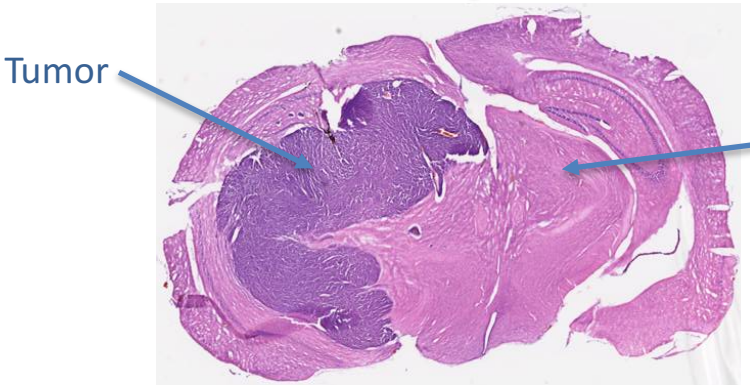
Brain sectioning and analysis:



500µm distance sections



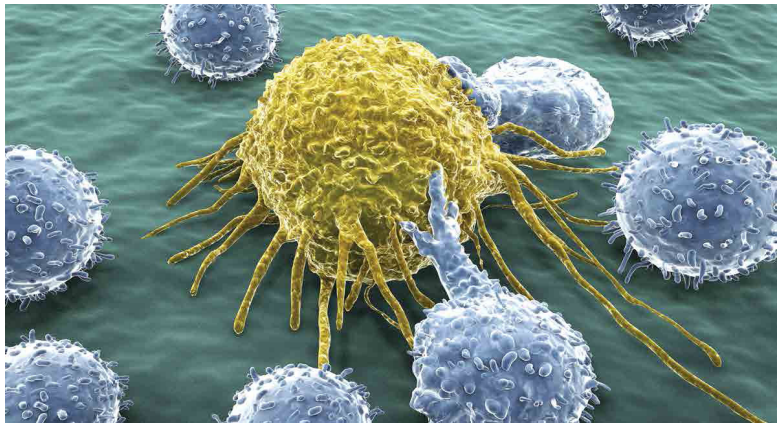
NIRF images of the brain sections

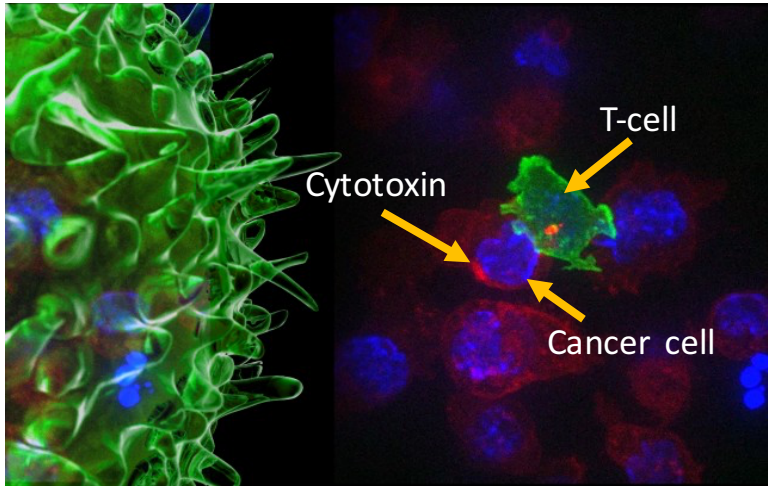


H & E stained tissues showing tumor margin

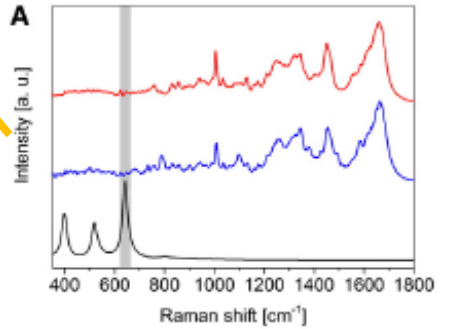
Immune Cells for Delivery of Contrast Agents and Therapeutic Nanoparticles to Brain Tumors:

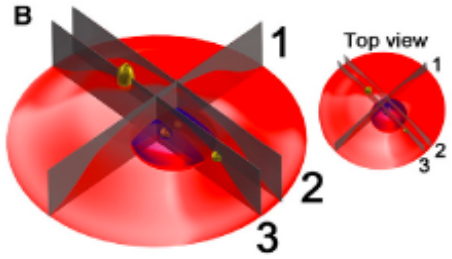
A cancer-killing cytotoxic T-cell

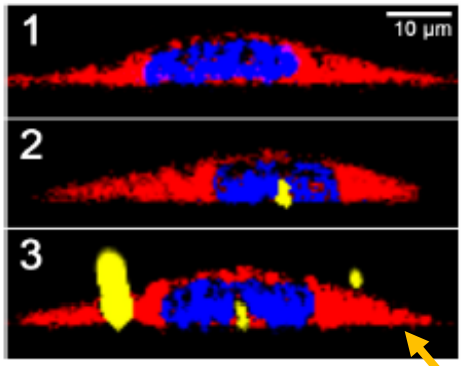




Spectra of cell cytoplasm (red), nucleus (blue) and TiO2 NPs (black)



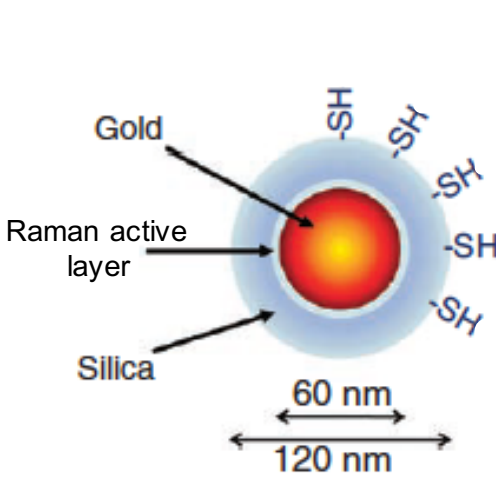




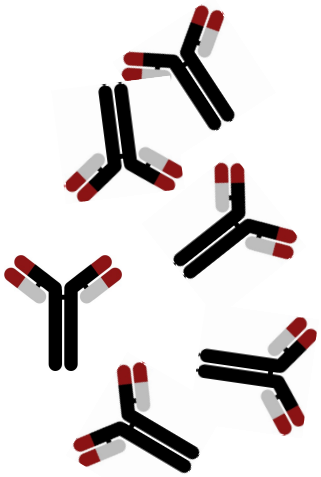
Cross section Raman images: cell cytoplasm (red), nucleus (blue) and TiO2 NPs (yellow)

B. Kann et al. Analyst 139 (2014) 5069–5074.

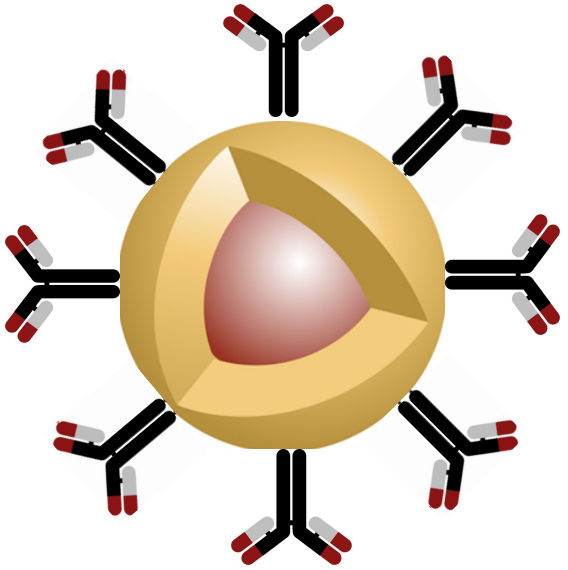
Preparation of the Raman nanoparticles for cell labeling:



Nanoparticles

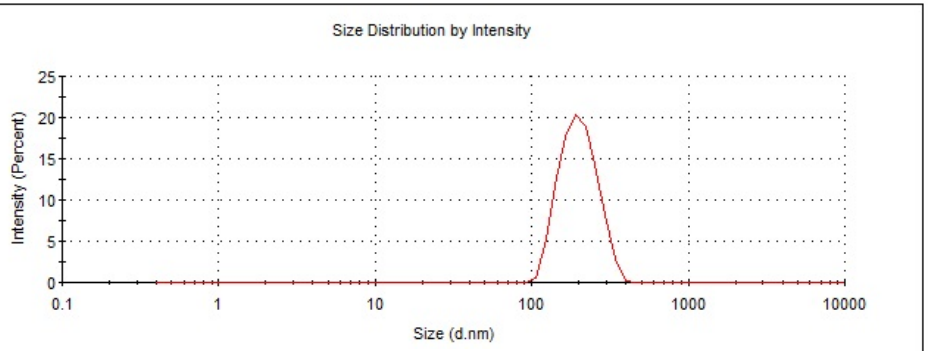


Anti-CD47

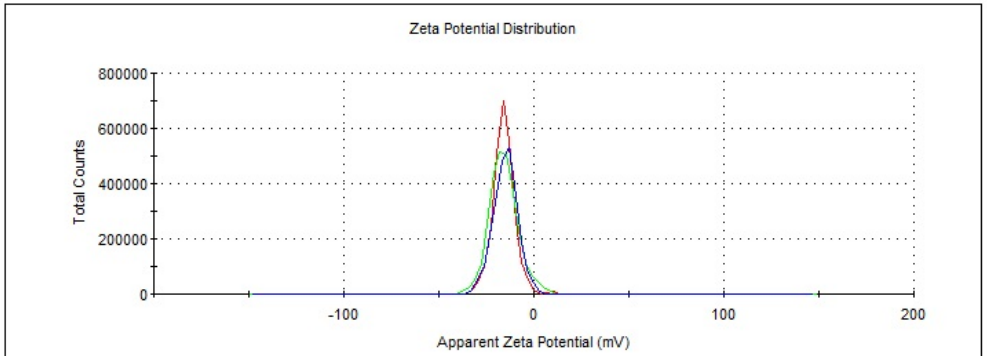


Antibody conjugated Raman Nanoparticles

Hydrodynamic size and surface charge of antibody conjugated Raman nanoparticles

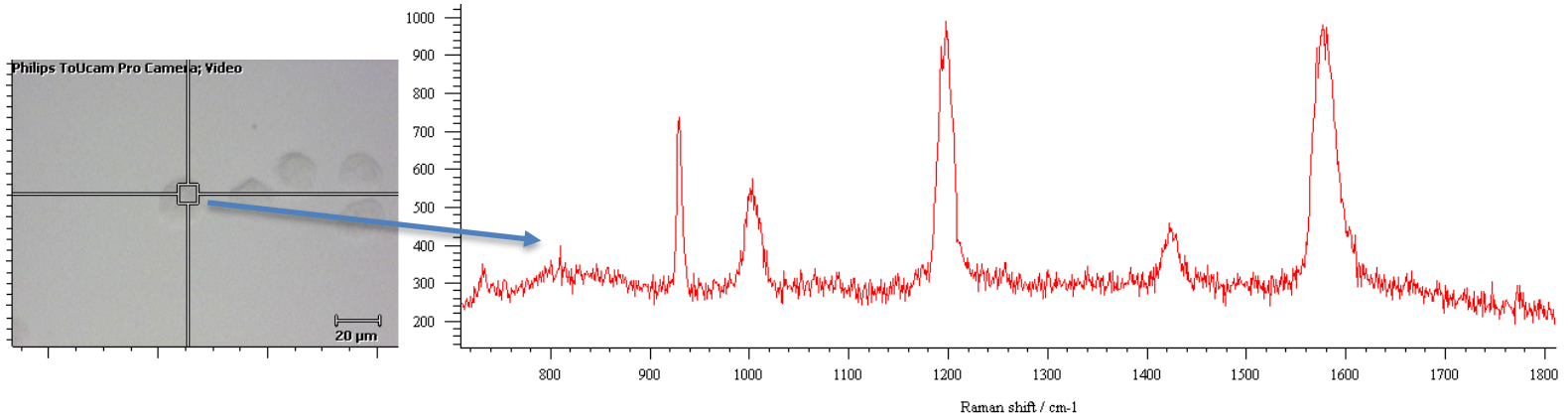


Average size= 189.1 nm; Polydispersity index (PDI)= 0.094

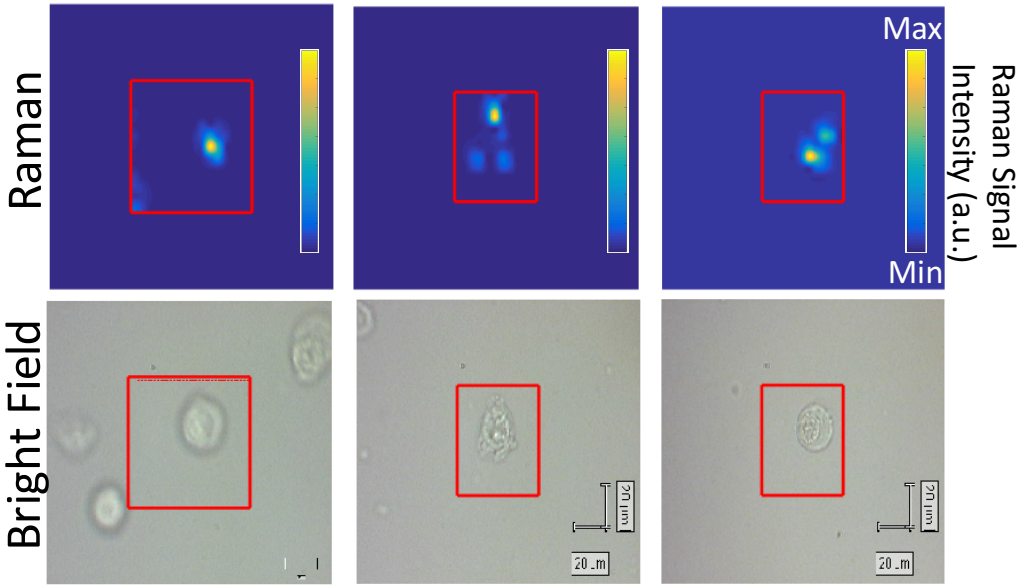


Zeta potential= -15.9 mV

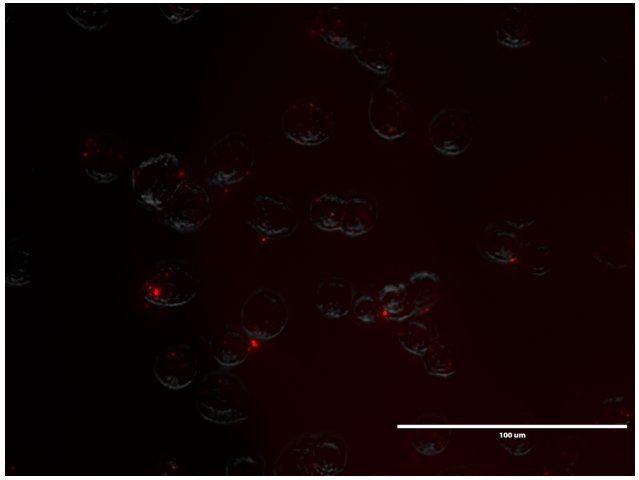
Raman and fluorescent imaging of the MCF7 breast cancer cells after nanoparticles binding:



Single cell Raman spectroscopy of the MCF7 cells after nanoparticles binding

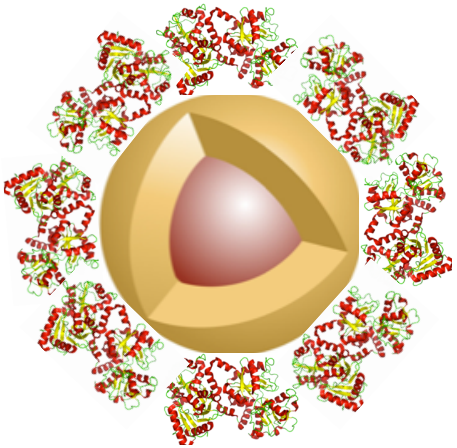


Single cell Raman images of the MCF7 cells after nanoparticles binding

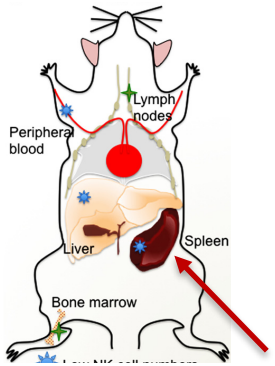


Fluorescent images of the MCF7 cells after nanoparticles binding

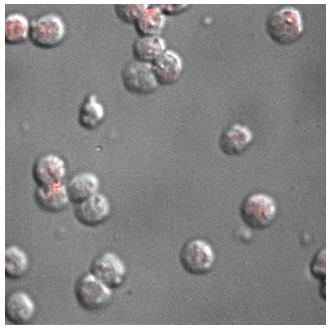
Labelled immune cells for nanoparticles delivery to brain tumors:



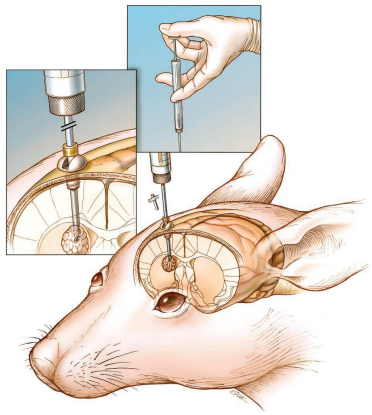
Preparation and Characterization of the Raman nanoparticles for high uptake



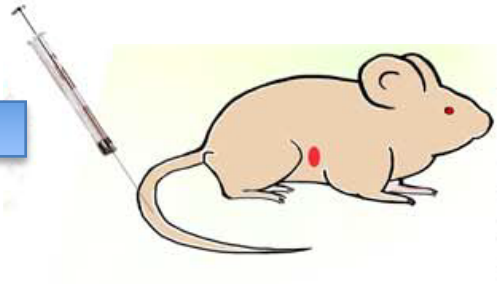
T-cell isolation from spleen and training them for specific brain tumors



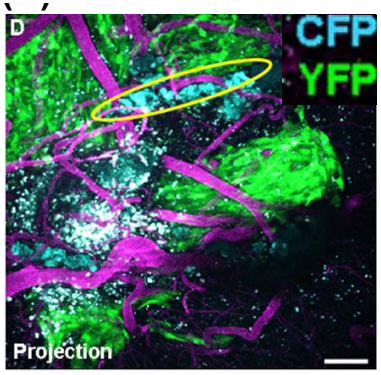
Labeling of the trained T-cells and analyses of the labelled cells



Intracranial implantation of the tumors

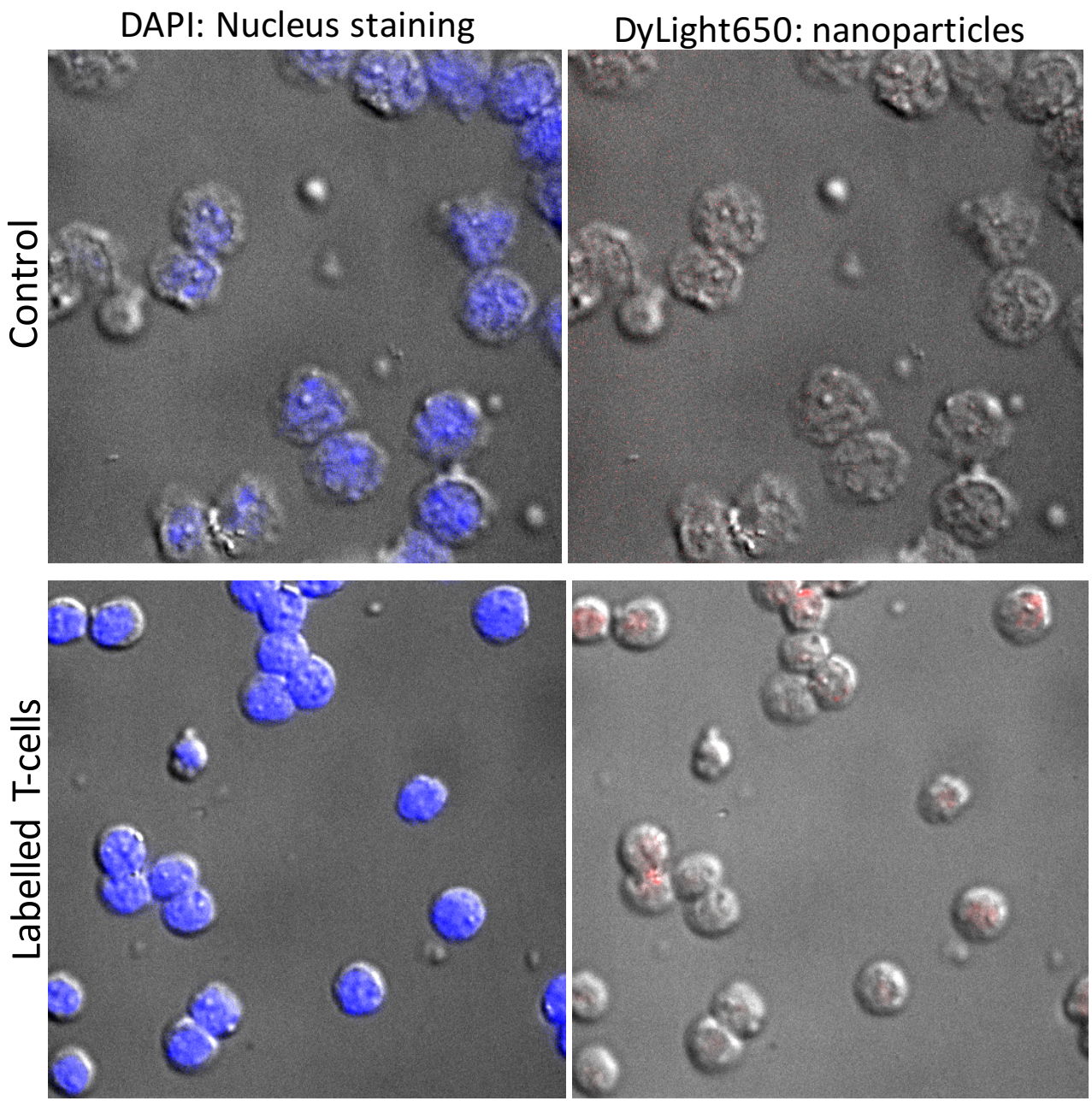


Administration of the labelled T-cells

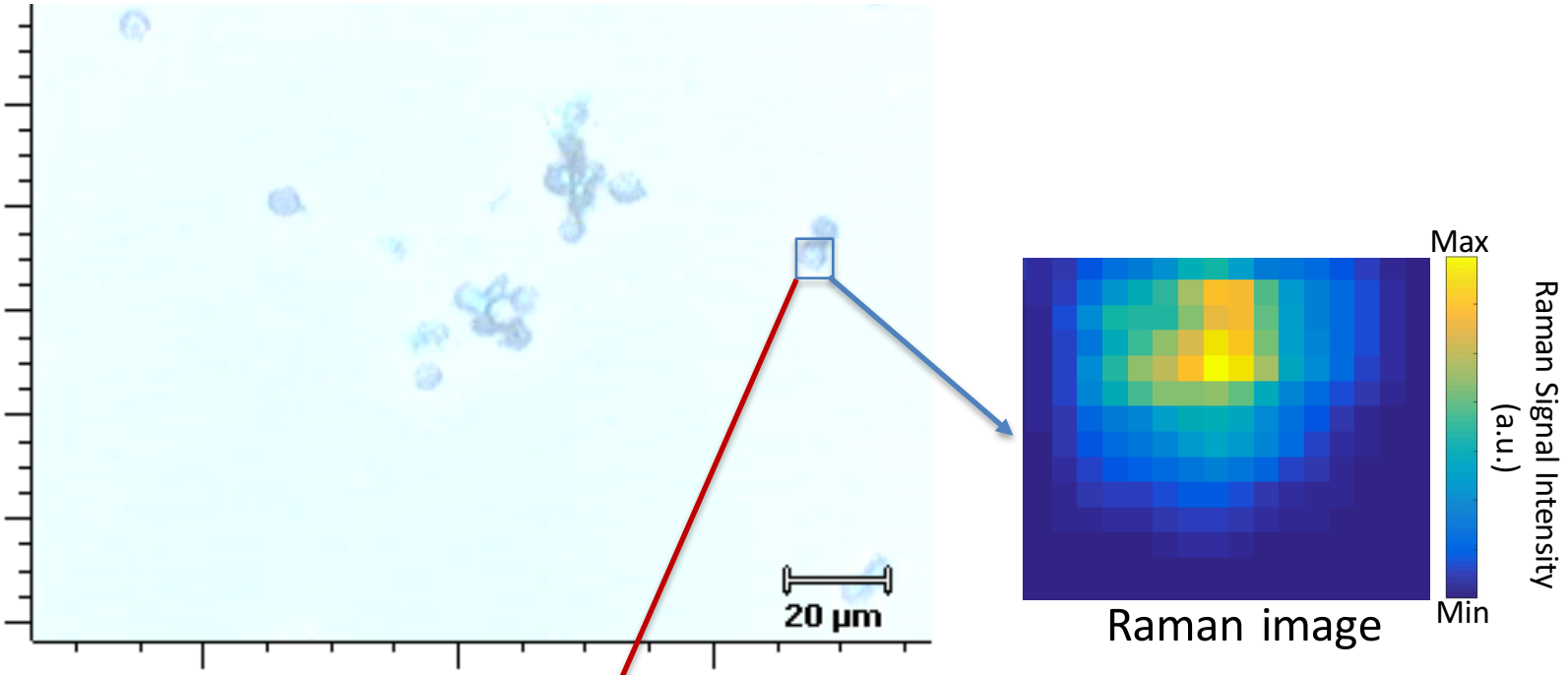


Brain Imaging,
Intravital microscopy, Histology

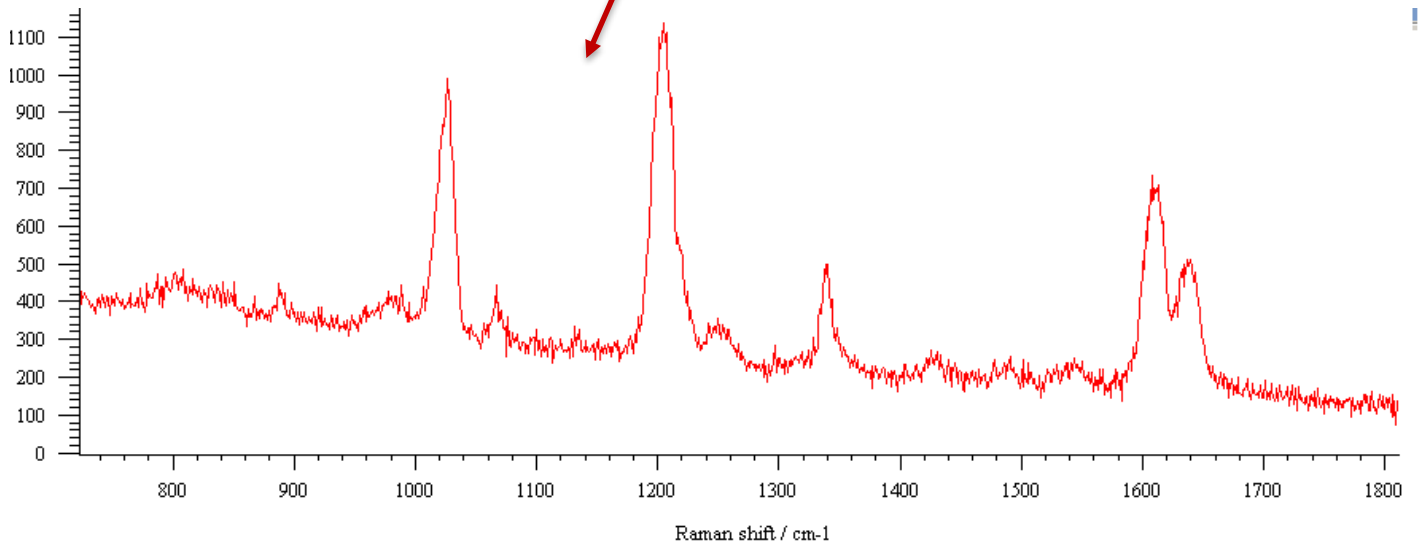
Fluorescent images of the naïve T-cells labelled with Raman nanoparticles:



Preliminary Raman image of a naïve T-cell labelled with Raman nanoparticles:



Raman image



Typical Raman spectrum on a selected point of a labelled T cell

Conclusions & Future Plans

- **Nanoparticles development:** PEG coating stabilized the NPs

- **Clinical studies:**
- Preliminary clinical results are promising. More cases will be studies.
- Large scale synthesis of nanoparticles

- **Intraoperative studies:**
- More mice brains are under investigations.
- Quantification of the temperature increase
- Performing the experiment *in vivo*

- **Immune cell studies:**
- Proliferation and functionality of the labelled T-cells should be studied more accurately.
- Different types of T-cells (CD8+ and CD4+ T-cells) will be tested, with specificity to our tumor model (GL26)
- Administration of the labelled T-cells to mice with tumor and in vivo imaging

Image: Raman nanoparticles diffused into the brain tumor

- Prof. Gambhir (Stanford, Radiology)
- Prof. Sinclair (Stanford, MSE)
- Edwin Chang, Chirag Patel, Steven Madsen, Ryan Davis, Nicole De Jesus, Jung Ho Yu, Travis Shaffer, Aaron Mayer, Demir Akin, Carmel Chan, Surya Murty
- Gambhir's lab
- Dr. Dickinson (UC Davis)
- Others

Stanford Cancer Imaging Training (NIH T32)

Molecular Imaging Program at Stanford (MIPS)

School of Medicine
Department of Radiology