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Thermal and mechanical effects of different excitation modes based on low frequency laser modulation in optical hyperthermia

Cristina Sánchez, PhD student
cristina.sanchez@ctb.upm.es

Laboratory of Bioinstrumentation and Nanomedicine (LBN-CTB)

www.ctb.upm.es

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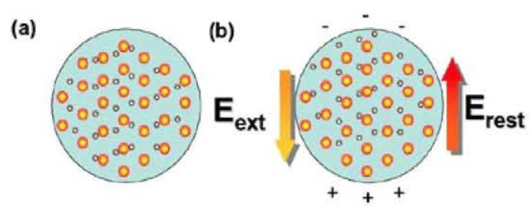
- Summary
  - Introduction
    - Metal nanoparticles and cancer
  - First results
    - Optical hyperthermia therapy (proof of concept): gold nanorods + continuous wave laser + glioblastoma cell lines (1321N1)
  - Present work
    - New excitation methods (low frequency TTL modulation)
    - Mechanical effects: measures and contribution to the cellular death
  - Future work
    - Optimization of hyperthermia therapy parameters (optical and mechanical)

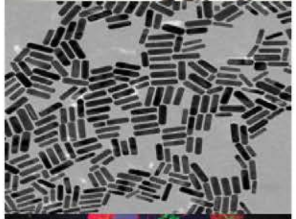
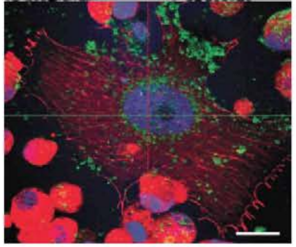
- Introduction: metal nanoparticles and cancer
  - The SPR effect in gold nanoparticles exposed to a laser source allows the development of hyperthermia therapies:
    - collective oscillation of free electrons
    - strong enhancement of light absorption
    - conversion of the absorbed light into localized heat



NPs in equilibrium (a) and NPs under application of an external magnetic field (b)

  - Main objective:
    - development and characterization of instrumentation to produce optical hyperthermia in biological samples

- First results: proof of concept
  - "Induction of cell death in a glioblastoma line by hyperthermic therapy based on gold nanorods" (Fernandez Cabada et al., Int. J. Nanomed 2012:7 1511–1523, JCR:4.9)
    - Material and methods:

- Hyperthermia device → CW laser (808 nm) + precision thermometer + optical instrumentation → optimal penetration in tissues
- Nanoparticles → commercial gold nanorods (Nanopartz A12-10-808) → efficient light absorption
- Cell cultures → human brain astrocytoma cells (1321N1)
- Hyperthermia treatments → cells incubated with and without gold nanorods were exposed to laser irradiation (controls were run in parallel)
- Analysis → immunocytochemistry, cell viability assays, uptake studies and statistical analysis

- First results: proof of concept (II)
  - "Induction of cell death in a glioblastoma line by hyperthermic therapy based on gold nanorods" (Fernandez Cabada et al., Int. J. Nanomed 2012:7 1511–1523, JCR:4.9)
    - Results:

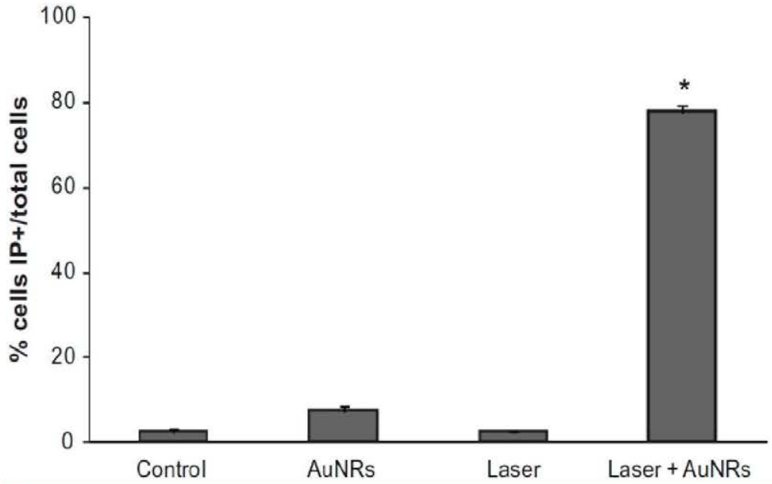


Figure 1: Flow cytometry analysis of 1321N1 cell death by labeling with propidium iodide after hyperthermia treatment. The graph show the percentages of dead cells (IP+-cells) over total cells

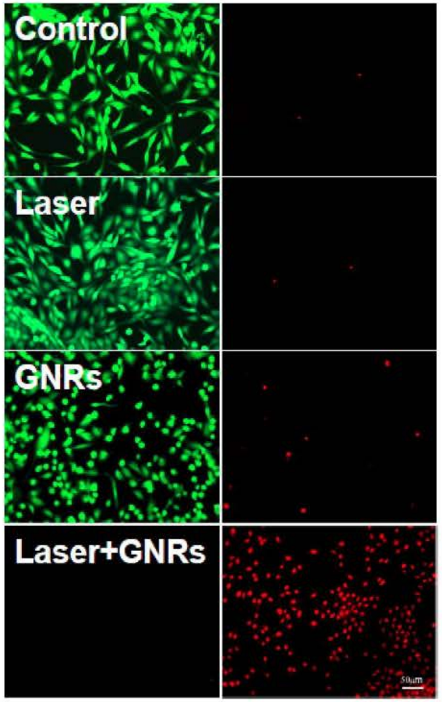


Figure 2: Cell viability after hyperthermia treatments. 1321N1 cells were stained with calcein and propidium iodide to visualize live (green) and dead (red) cells, respectively

- First results: proof of concept (III)
  - "Induction of cell death in a glioblastoma line by hyperthermic therapy based on gold nanorods" (Fernandez Cabada et al., Int. J. Nanomed 2012:7 1511–1523, JCR:4.9)
    - Conclusion:
      - The use of gold nanorods in hyperthermal therapy is very effective and an important area of research and therapeutic development
      - Gold nanorod surfaces need further modification to make them biocompatible and suitable for imaging and as theragnostic agents for clinical purposes
      - It is important the use of chemically stable coatings other than CTAB to avoid the cytotoxic effect observed
      - Due to the limited capacity of laser penetration in tissues, the method could provice an additional aid to surgery for removing brain tumors

• Present work: new excitation methods

– Does the low frequency (< 30KHz) TTL modulation of the laser influence on the cellular death?

• Temperature curves → show the same thermal behavior in continuous wave and modulation modes

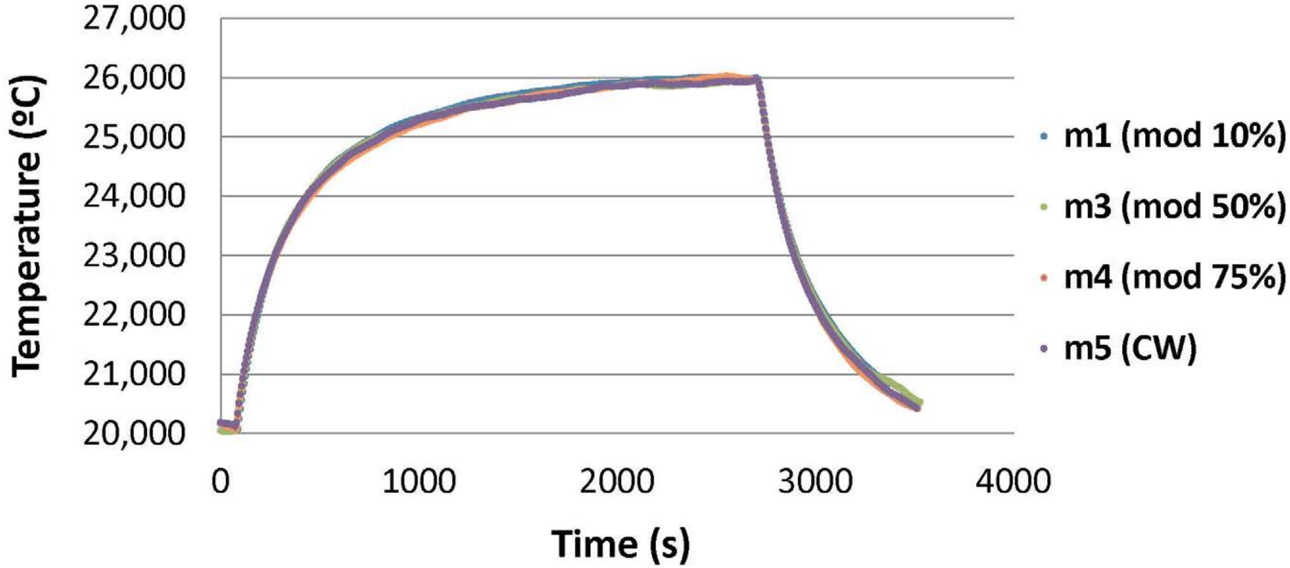


Figure 3. Temperature curves of gold nanorods suspension for different duty cycles of modulation in comparison to the continuous wave mode (CW). The parameters of the laser are fixed in an average power of 381 mW and a frequency of modulation of 5KHz

• Present work: new excitation methods (II)

– Does the low frequency (< 30KHz) TTL modulation of the laser influence on the cellular death?

• Cellular death (preliminary results) → the higher is the frequency the higher is the percentage of dead cells → are there other effects that contribute to the cellular death?

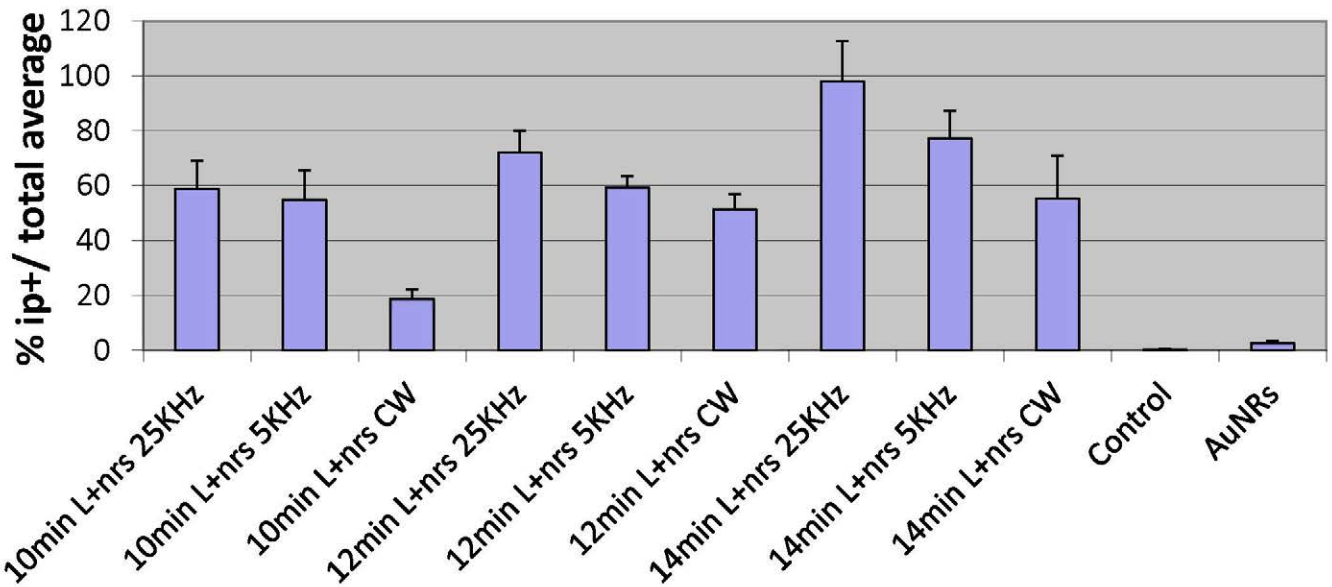


Figure 4. IP/calcein essay 24h after irradiation (3W): Comparison between different times and excitation modes (modulation and CW).

- Present work: mechanical effects
  - What kind of mechanical effects are expected? → sound/pressure waves (due to the thermal expansion of gold nanorods)
  - What is the behaviour and magnitude of these processes? → development of a measure device based on:
    - Ultrasound piezoelectric receivers (25KHz)
    - Lock-in amplifier → is able to detect sound waves generated in samples of gold nanorods during laser irradiation

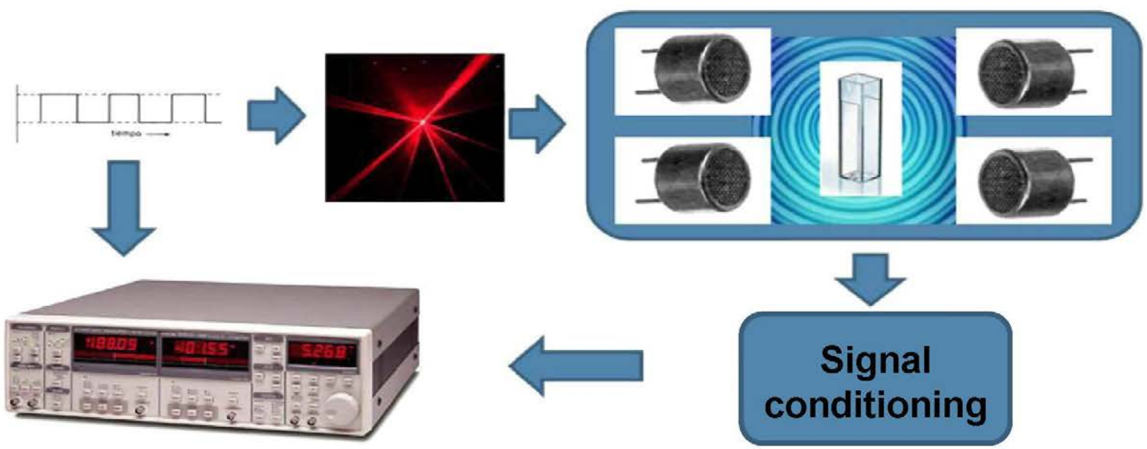


Figure 5. Ultrasound measure device

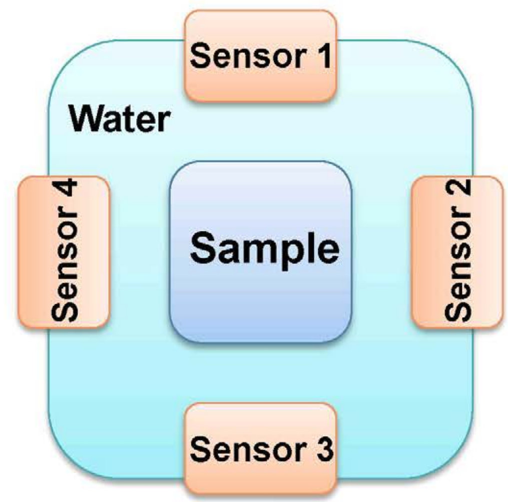


Figure 6. Experimental structure diagram

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- Present work: mechanical effects (II)
  - Results → the pressure measurements are directly proportional to the concentration of gold nanorods and the laser power and inversely proportional to the duty cycle

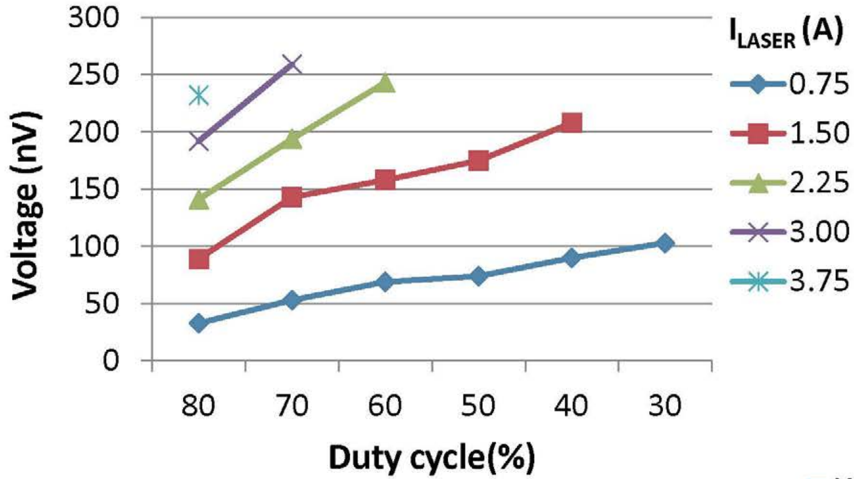

Figure 7. Voltage levels for different laser intensity values (linearly proportional to the power source) in a duty cycle sweep (36µg/ml, sensor 3)

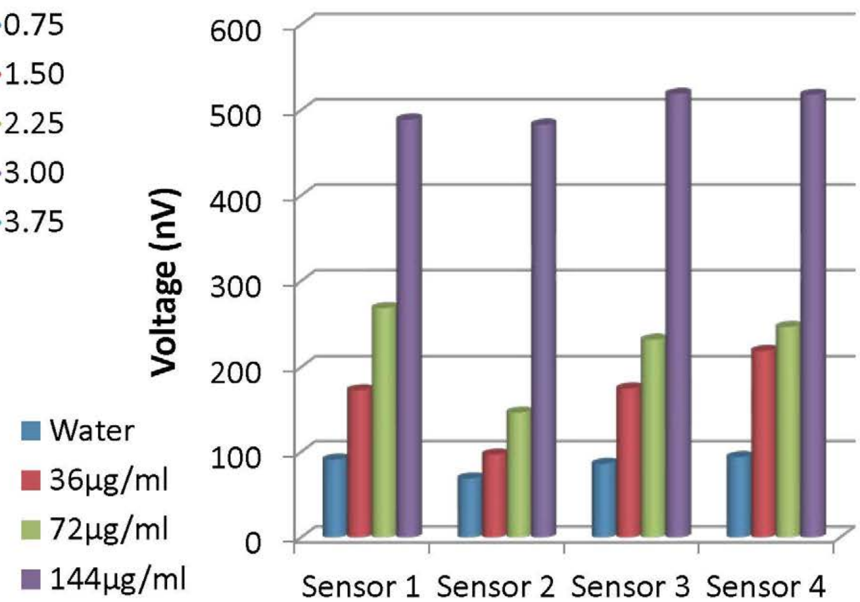

Figure 8. Voltage levels for different values of gold nanorods concentration (50% duty cycle, 1.5A)

- Future work

  - New experiments of modulation → exhaustive study of the influence of the modulation parameters on the cellular death processes → statistical analysis
  - The ultrasound measure device needs to be improved in order to establish an univocal relation between the system parameters (laser power, concentration of gold nanorods, frequency of TTL modulation…) and the detected pressure/sound signals and their magnitude
  - It is important to determine if when the temperature is not high enough to produce cellular death (<42ºC), the mechanical effects are able by themselves to produce cellular damage or even death
  - For effective hyperthermia treatment, many parameters still need to be optimized, concerning both laser and gold nanorods, in order to achieve treatments that work as effectively as possible

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Thank you!

"The important thing is not to stop questioning.
Curiosity has its own reason for existing."
Albert Einstein

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