Two-photon polymerization of Proteinaceous microstructures with Near-infrared photothermal activity

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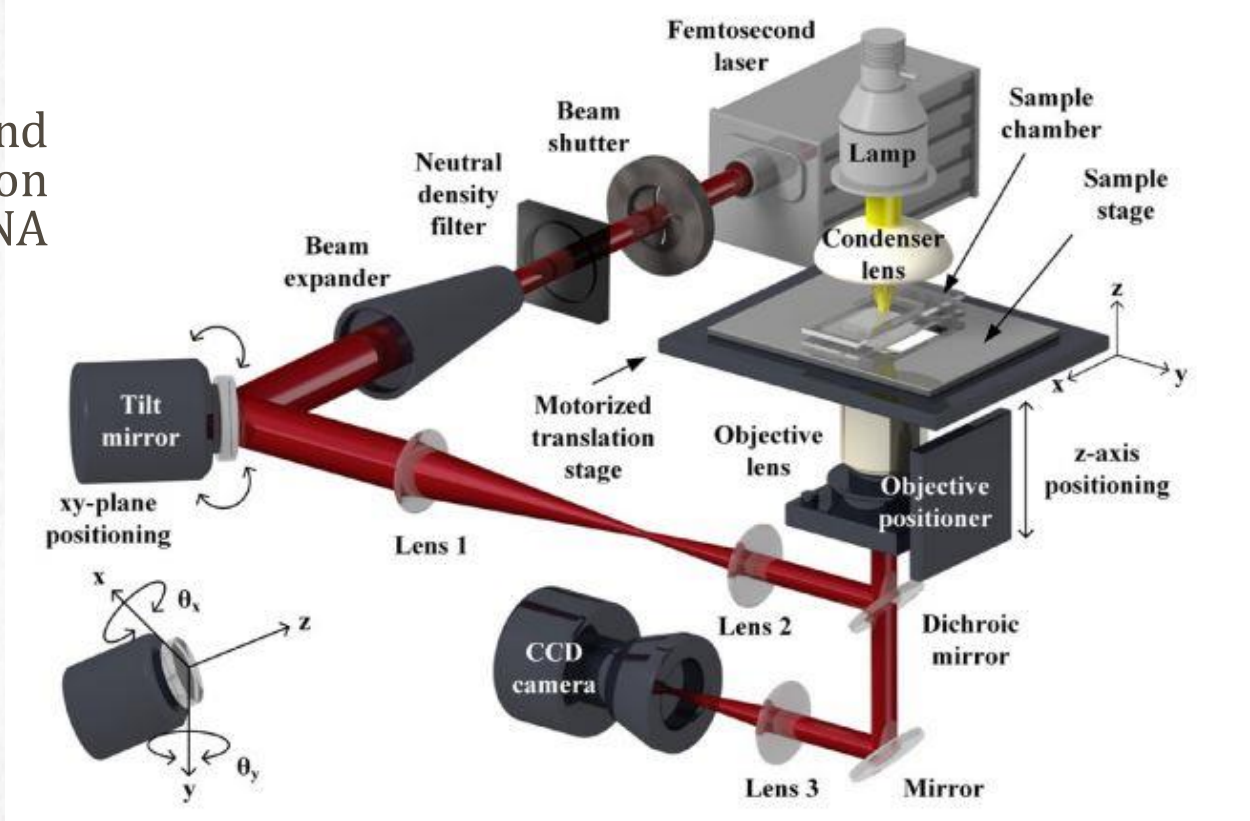
AGENDA

- **Direct Laser Writing (DLW)**
  - Methylene Blue (Track 1)
  - Rose Bengal (Track 2)
  - Rose Bengal + Gold NanoStars (Track 3)
- **Photo-Thermal Activity**
- **Ongoing and Future Perspectives**

DLW, TWO-PHOTON SETUP

- Tsunami Series; Ti:Sapphire femtosecond pulsed laser, mounted on an inverted Nikon microscope with the AI objective (60X, NA 0.85); XYZ piezo-driven stage
  - Laser repetition rate; 80 MHz
  - Pulse duration; 200 fs
  - Working wavelengths; 780-820nm

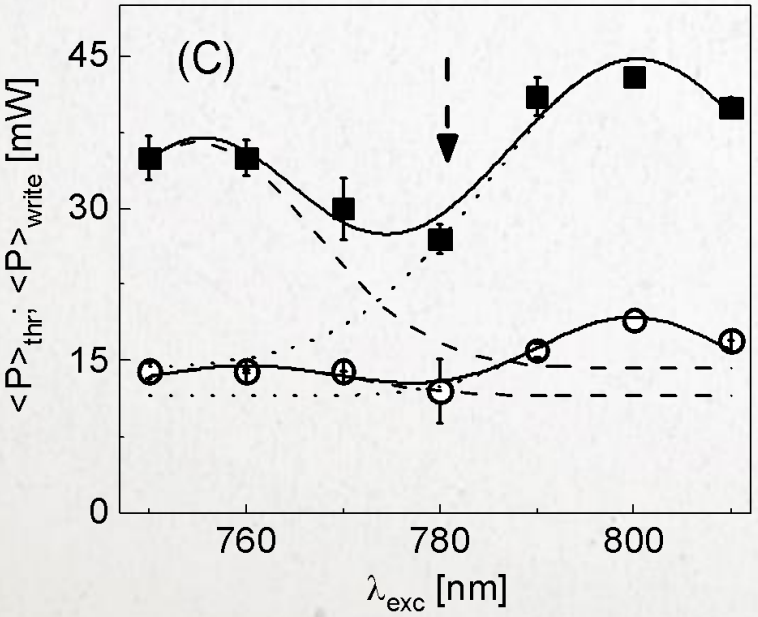


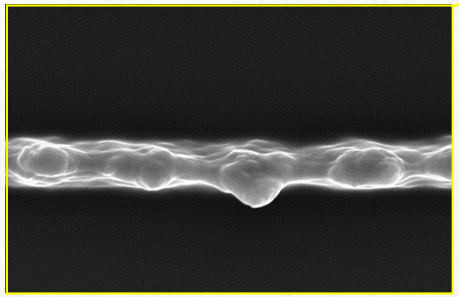
TWO-PHOTON CROSSLINKING (TPC), TRACK 1

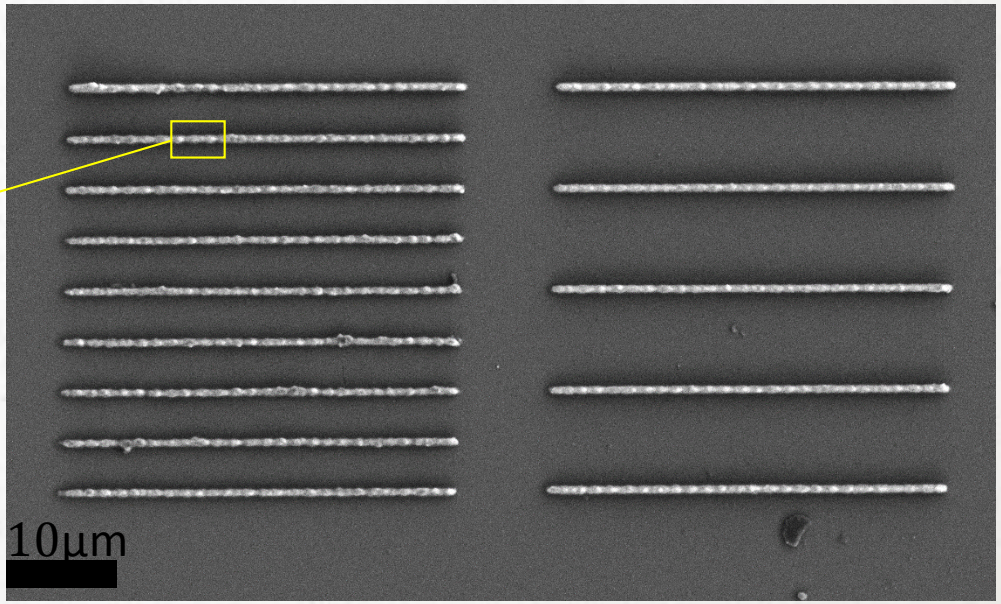
- Bovine Serum Albumin (BSA) and Methylene Blue (MB,)

  Source Working wavelength; 780nm

MB excitation band 633nm.
Following rule of λ/0.7 the two-photon peak locates around ~900nm





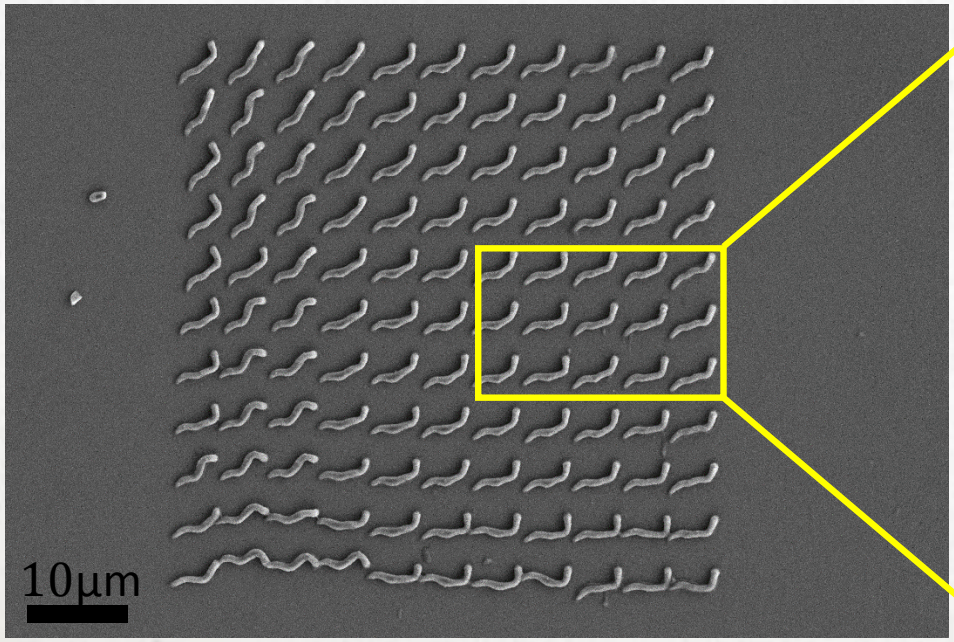


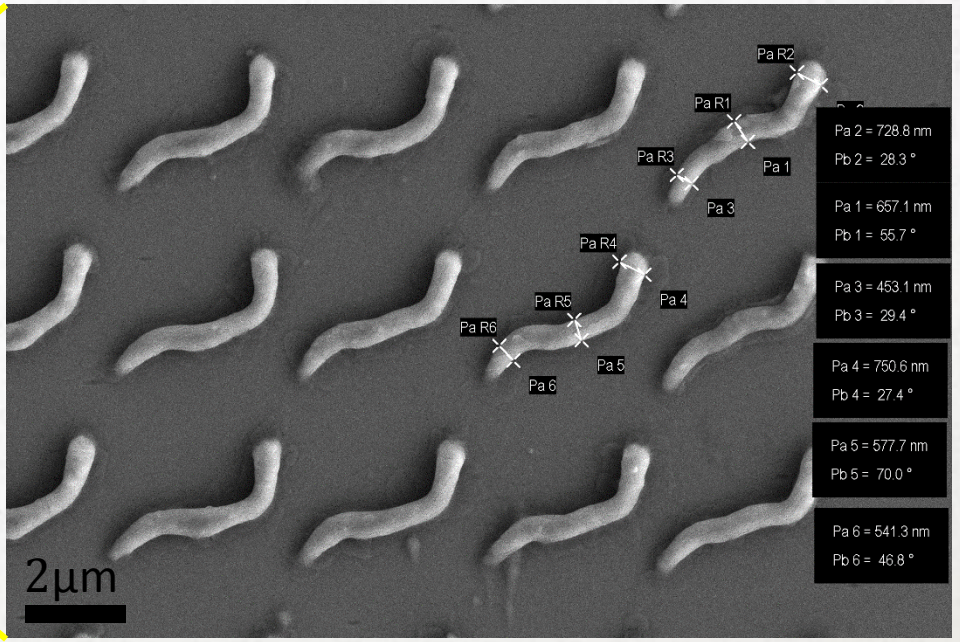
TWO-PHOTON CROSSLINKING (TPC), TRACK 1

- Bovine Serum Albumin (BSA) and Methylene Blue (MB)

  Source Working wavelengths; 780nm

$E_{MB} = 240 \pm 80\ kPa$



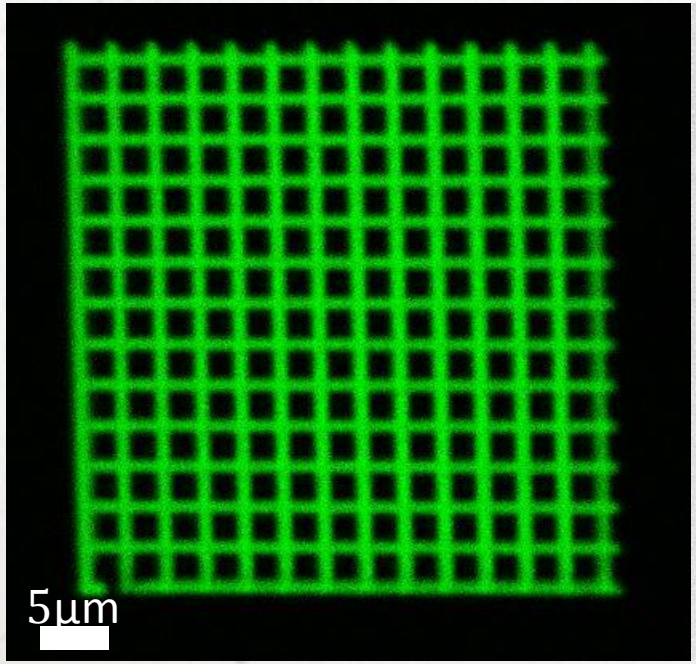


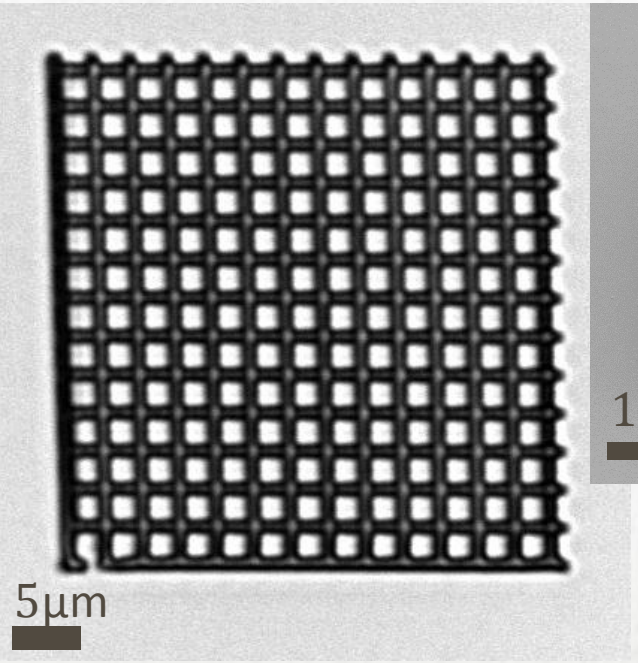
TWO-PHOTON CROSSLINKING (TPC), TRACK 2

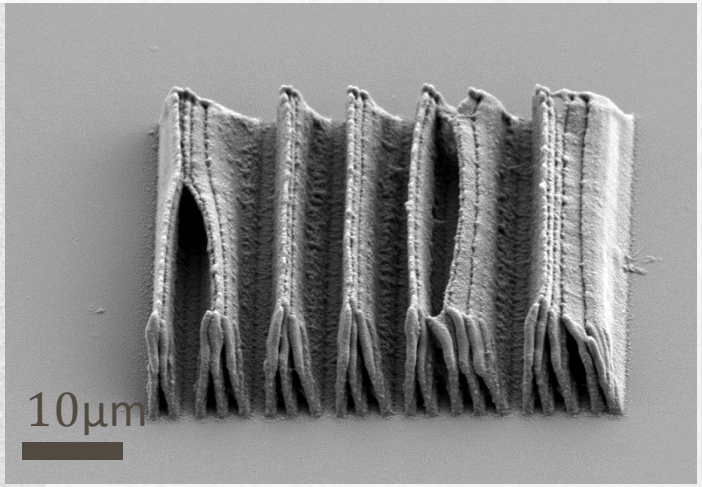
- Bovine Serum Albumin (BSA) and Rose Bengal (RB)

  Source Working wavelengths; 800nm

RB excitation band 514nm.
Following rule of $\lambda/0.7$ the two-photon peak locates exactly at 800nm







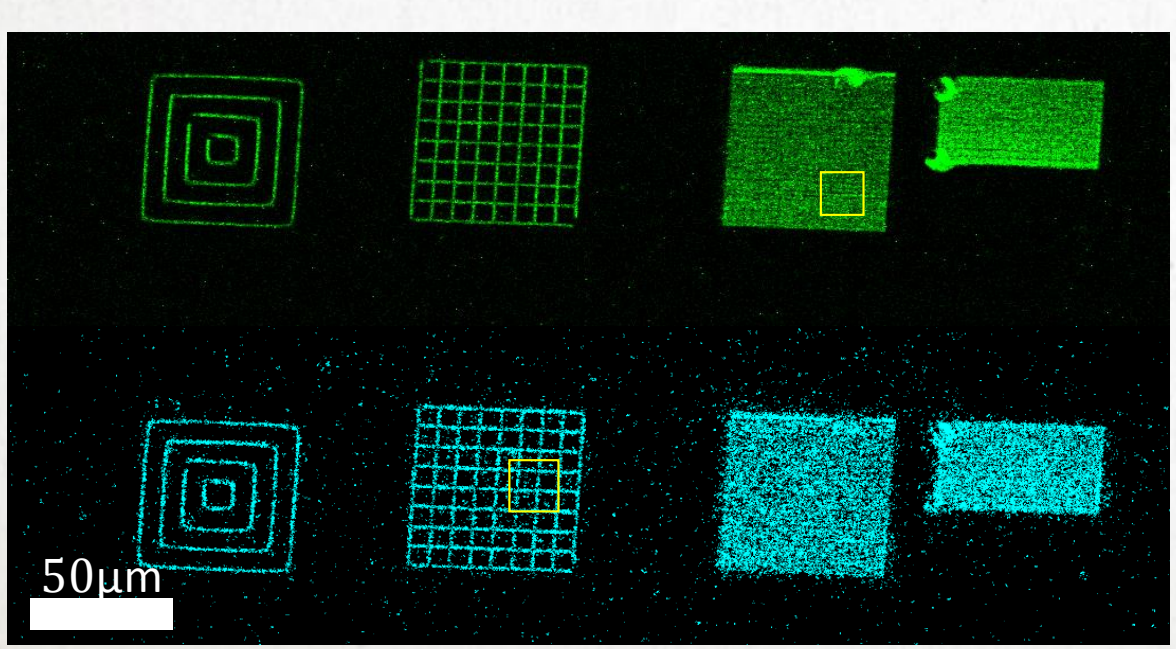
$$E_{RB} = 820 \pm 300\ kPa$$

TWO-PHOTON CROSSLINKING (TPC), TRACK 3

(A1)

- Bovine Serum Albumin (BSA), Rose Bengal (RB), and Gold Nanoparticles (GNS)

(A2) *Green channel (RB emission 550nm) – Cyan channel (GNS Two-photon luminescence)*



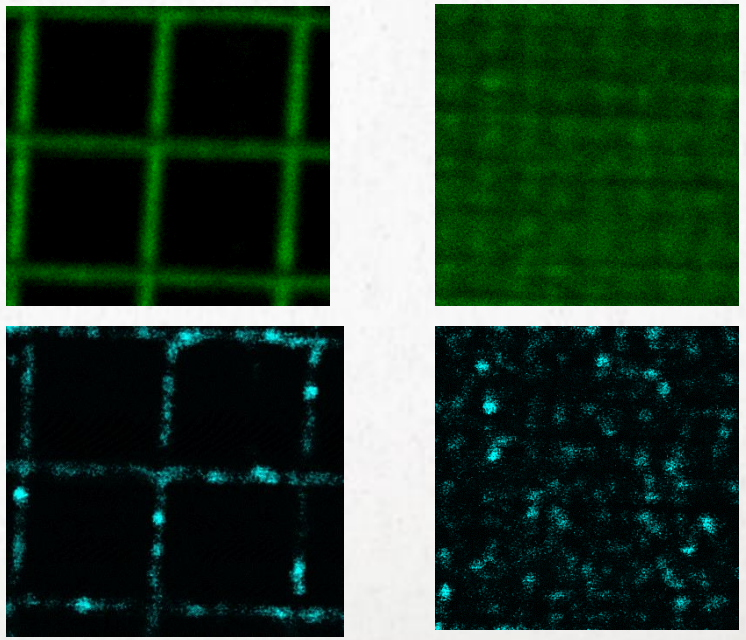
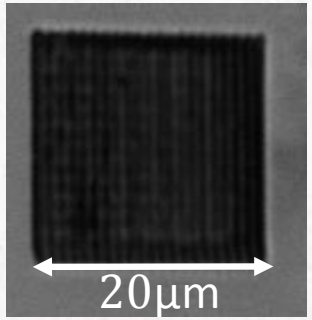
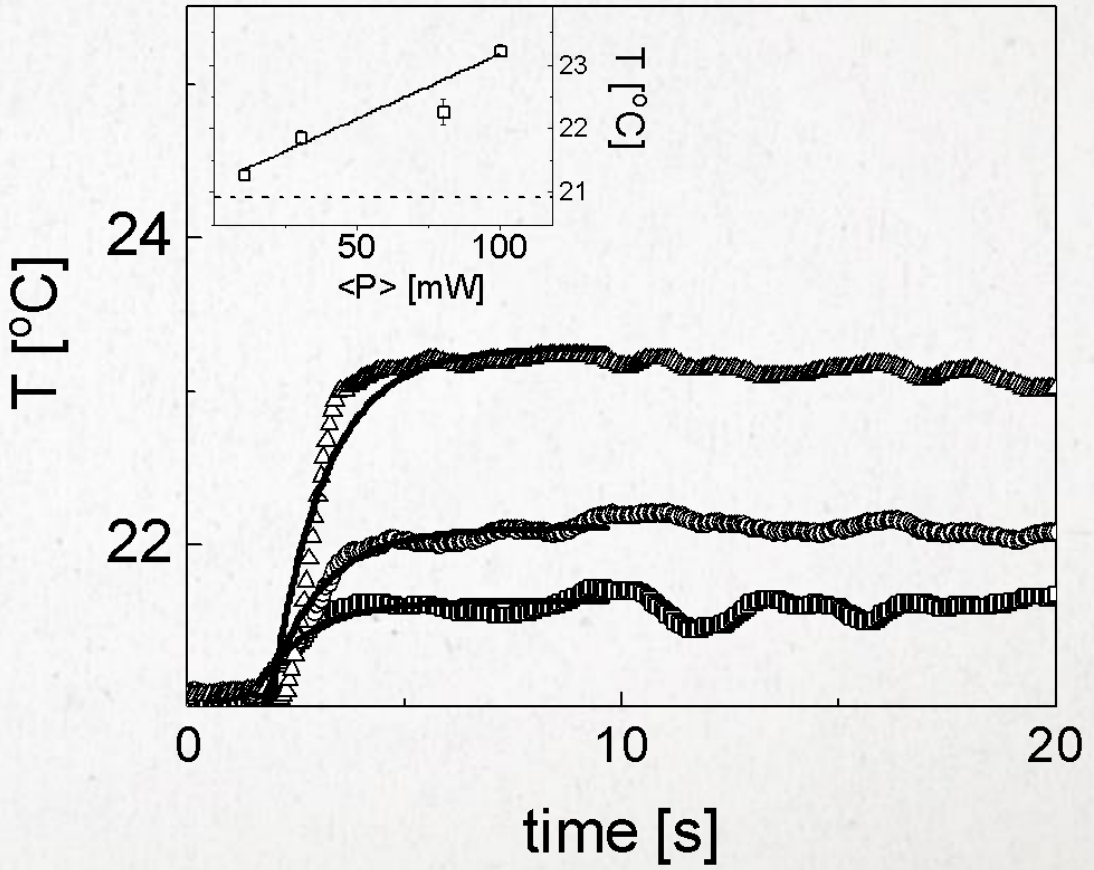


PHOTO-THERMAL EFFECT

- Tested on a uniform filled BSA/RB/GNS
- Under continuous irradiation of NIR pulsed laser (800nm)

- <P> up triangles = 100mW
  <P> circles = 80mW
  <P> squares = 30mW

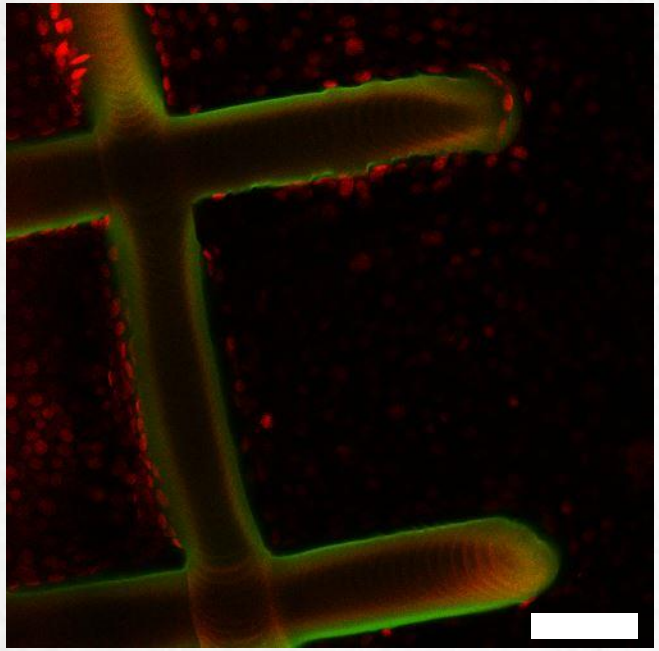




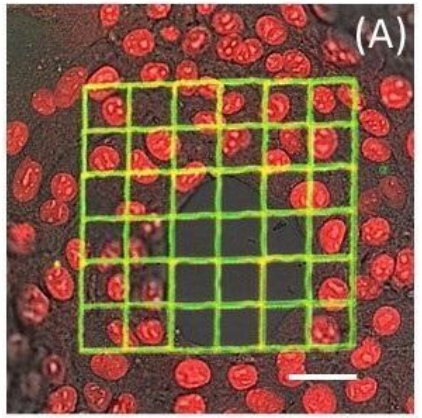
Recording device was a FLIR E40 thermocamera

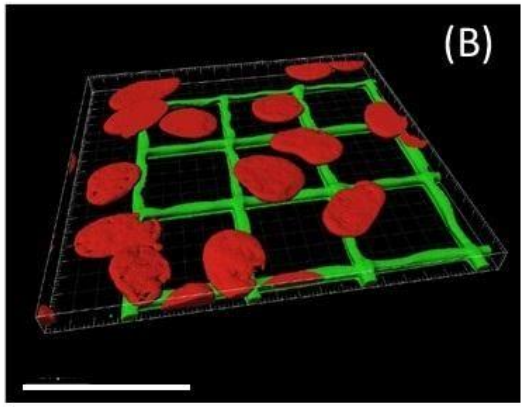
Bouzin, M., Marini, M., Zeynali, A., Borzenkov, M., Sironi, L., D'Alfonso, L., Mingozzi, F., Granucci, F., Pallavicini, P., Chirico, G. and Collini, M., 2019. "Photo-activated raster scanning thermal imaging at sub-diffraction resolution". *Nature Communications*, 10(1).

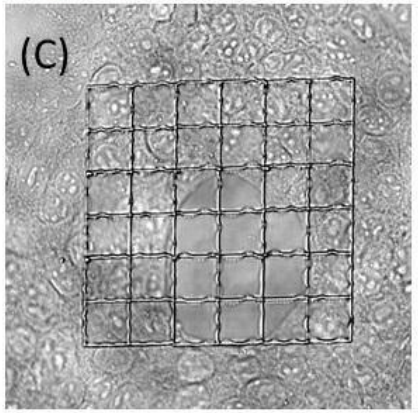
ONGOING AND FUTURE PERSPECTIVES



NIH 3T3 fibroblasts cell line
[Propidium iodide; red fluorescence
Rose Bengal; green channel]
Scale bar 50µm







(Nuclei stained with Draq5)
Scale bar 10µm

The triple negative breast cancer 4T1

Zeynali, A., Marini, M., Chirico, G., Bouzin, M., Borzenkov, M., Sironi, L., D'Alfonso, L., Pallavicini, P., Cassina, V., Mantegazza, F., Granucci, F., Marongiu, L., Polli, D., De la Cadena, A. and Collini, M., 2020. **"Multiphoton Fabrication of Proteinaceous Nanocomposite Microstructures with Photothermal Activity in the Infrared".** *Advanced Optical Materials*, p.2000584.

THANK YOU FOR YOUR ATTENTION