MICRO-ET NANO-CRISTAUX DE MOLÉCULES ORGANIQUES POUR DES APPLICATIONS BIOMÉDICALES : REPRÉCIPITATION PAR CAVITATION ET ONDE DE CHOC, FORMULATION, TESTS BIOLOGIQUES, IMAGERIE DE FLUORESCENCE ET IMAGERIE PHOTOACOUSTIQUE

MICRO- AND NANO-CRYSTALS OF ORGANIC MOLECULES FOR BIOMEDICAL APPLICATIONS: CAVITATION AND SHOCK WAVE REPRECIPITATION, FORMULATION, BIOASSAYS, FLUORESCENCE IMAGING AND PHOTOACOUSTIC IMAGING

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The research project will investigate the feasibility of the laser-induced method for artificial crystallization of organic dye compounds. Crystals of organic dye materials have been utilized as optical elements for several kinds of devices, such as a light-modulating material, optical recording media, optical switching material, and functional ink. Especially photochromic and luminescent crystals have attracted much attention for their high functionalities. The PhD work will focus on chromophores that can be luminescent or not, their final application is either photoacoustic imaging (for non-luminescent crystals) or luminescence microscopy. Luminescent materials are sought for their properties as light-emitters, and sensors. Considering application to industries, it is important to establish a mass-production process to fabricate same crystals with uniform qualities, shapes and phases. However, it is very difficult to control them with high reproducibility.

Photoacoustic imaging (PAI) is an emerging biomedical imaging modality[1] combining optical and ultrasound waves to map optical-absorption contrast at centimetric depth in sub-millimeter resolution. The key is the photoacoustic (PA) effect: optically absorbing structures emit ultrasounds (thermoelastic expansion) when excited with a nanosecond laser pulse (Figure 1). To reach centimetric depth, PAI operates in the near-infrared (NIR) window in biological tissue (650-1000nm), and uses medical ultrasound detectors. Separation of absorbers with different absorption spectra can be obtained using multispectral analysis (Multispectral PAI): sequential acquisition of PA images at different optical wavelengths, followed by spectral unmixing.[2] PAI is non-invasive, non-ionizing, cheaper than Magnetic Resonance Imaging (MRI)

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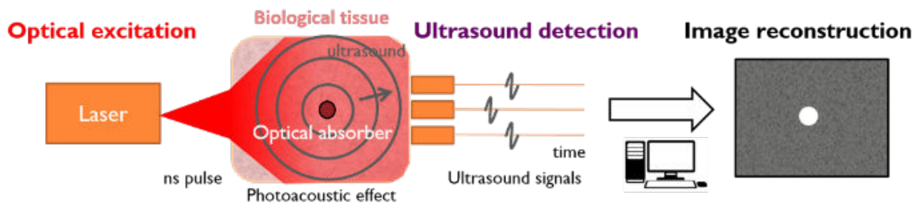
[1] Beard, P. Interface Focus 2011, 1 (4), 602–631

[2] Taruttis, A.; Ntziachristos, V. Nat. Photonics 2015, 9 (4), 219–227

and provides a sensitive detection of molecular agents[3] or nanoparticles.

Figure 1 : principle of photoacoustic imaging



NAIST group (Pr. HOSOKAWA, Dr. TSURI) has developed a crystallization technique using pulsed laser and succeeded in highly reproducible crystallization of various materials such as proteins and pharmaceutical compounds (Figure 2)[4]. In this technique, they induce artificial crystallization, in which crystal structure is highly controlled. As crystal structure with different crystal phase has different physical and chemical properties such as solubility and stability, control of crystal structure is crucial. Meanwhile, it is still challenging to obtain crystals with same desired structure, even with the optimization of environmental parameters such as concentration, temperature and solvents.

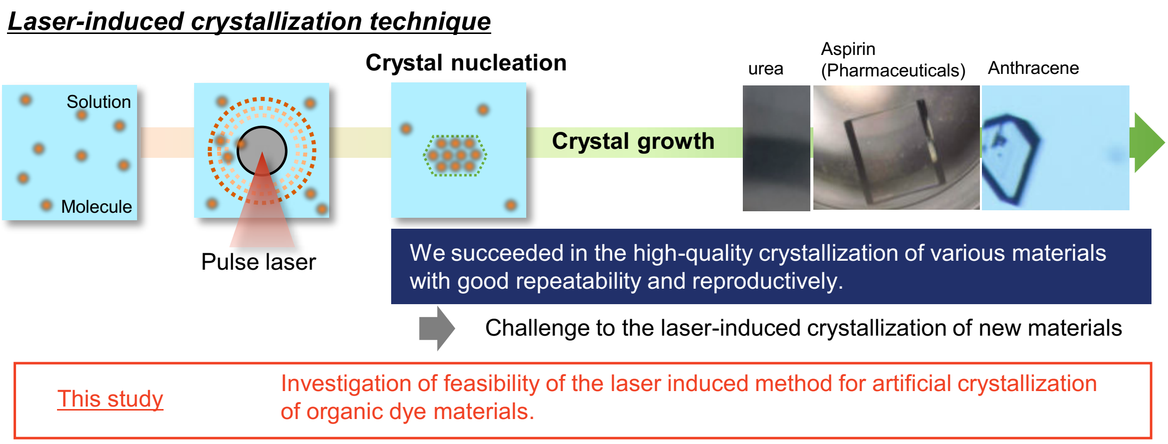


Figure 2: NAIST expertise and added-value to the PhD ADI proposal.

In the laser-induced crystallization NAIST partners has developed, the crystal nucleation is controlled by cavitation bubble, shockwave, and stresswave with laser-induced breakdown of liquid.[5] Recently NAIST partners found that this crystallization method realizes to control crystal structures of pharmaceutical compounds that potentially show better pharmaceutical effects. It is more controllable compared with conventional crystallization methods based on adjusting environmental parameters[6]. NAIST partners confirmed that cavitation bubble, which is transient vapor bubble generated by the laser irradiation, is a key driving force for the crystallization. The cavitation bubble presumably increases concentration (supersaturation) and/or enhances heterogeneous nucleation at its air–liquid interface. **The PhD candidate will investigate these effects in aqueous solutions, the laser generates cavitation bubble in any solvents.**

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[3] Weber, J.; Beard, P. C.; Bohndiek, S. E. *Nat. Methods* **2016**, *13* (8), 639–650

[4] H. Adachi et al., Jpn. J. Appl. Phys. **43**, L1376 (2004)

[5] H. Y. Yoshikawa et al., Chem. Soc. Rev., **43**, 2147 (2014) - Y. Tsuri et al., Appl. Phys. A, **128**, 803 (2022).

[6] Y. Tsuri et al., Appl. Phys. Express, **12**, 015507 (2019).

As a next challenge, the PhD will aim to control crystallization of various materials in various solvents to obtain the desired crystal structure, he or she will focus on family of dyes that ISMO knows well, namely BODIPY and AZA_BODIPY dyes. BODIPY is a short name for the well-known boron dipyrromethene family, and it is generally used in biological researches (Figure 3).

These chromophores are biocompatible pigments. Most of them are luminescent, but lose their emission properties when gathered in condensed phase.[7] ISMO group has already a wide expertise in BODIPY photophysics as well as formulation as polymer nanoparticles either for bacteria detection[8] or for *in-vivo* photoacoustic imaging[9]. **In order to improve the brightness of luminescent nano-probes, it is of wide interest to crystallize BODIPY and preserve its luminescence. If the emission properties are lost, then such nanocrystals could be of high added-value for photoacoustic imaging.**

Thus high-quality and reproducible crystallization of BODIPY will open up new possibilities for its application (if luminescent for confocal fluorescence microscopy and if non luminescent for photoacoustic imaging). Moreover, the PhD candidate will benefit from the Centre de Photonique pour la Biologie et les Matériaux CPBM (managed by Prof. Méallet) to explore possibilities for innovative experiments (laser-induced shockwave within milli or microfluidics).

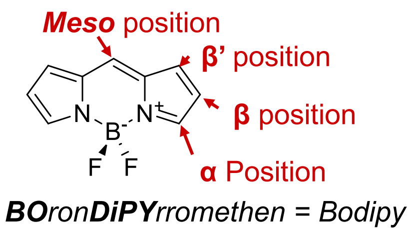

Figure 3 : BODIPY chromophore backbone

As a summary, the PhD candidate will investigate the feasibility of the laser induced method for artificial crystallization of organic dye materials, mainly BODIPY chromophores from ISMO.
Institut des Sciences Moléculaires d'Orsay (ISMO) -UMR 8214 – is located in Université Paris-Saclay. The laboratory combines the expertise of physicists and physical-chemists. This project will benefit from a recently acquired UV-vis spectrophotometer equipped with an integration sphere (280-1200nm) and a fluorimeter equipped with an integration sphere, polarization set-up and a lifetime module. A multimodal fluorescence microscopy platform is also accessible in CPBM (Centre de Photonique Biomédicale (FLIM, FRET, time lapse, FRAP, FCS which range for excitation and emission will be extended from UV to NIR (SESAME grant Ile de France). A chemistry-biochemistry, a P1 and P2-labs are accessible for easy handling and preparation of biological material will allow the PhD candidate to study the nano-crystals interactions with eukaryotic cells or bacteria (toxicity and growth tests).
He or she will also have the opportunity to participate to the IRP Nanosynergetics workshops.

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[7] T.T. Vu et al. *J. Phys. Chem. C* , 117, 10, 5373–5385 (2013).

[8] M. M Pan, et al. ACS Applied Polymer Materials 4 (8), 5482-5492 (2022)

[9] JB Bodin, et al. ACS Applied Materials & Interfaces 14 (36), 40501-40512 (2022)