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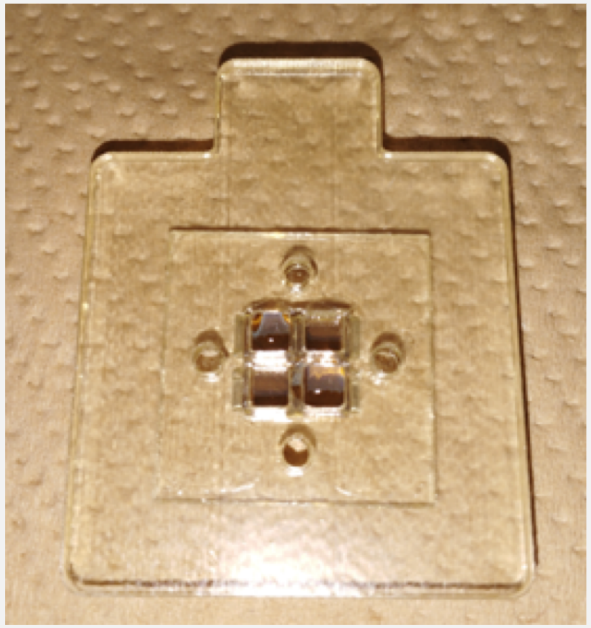
岡山大学 OKAYAMA UNIVERSITY

Upgrading THz microscopies for ultra fast medical imaging

Focus on breast cancer detection- Design of an optimized holder

A 4 months research internship in the group of
Pr. T. KIWA@ Okayama University in JAPAN

From May to September 2022



Tanguy DUSSON / Master 1st year (M1)

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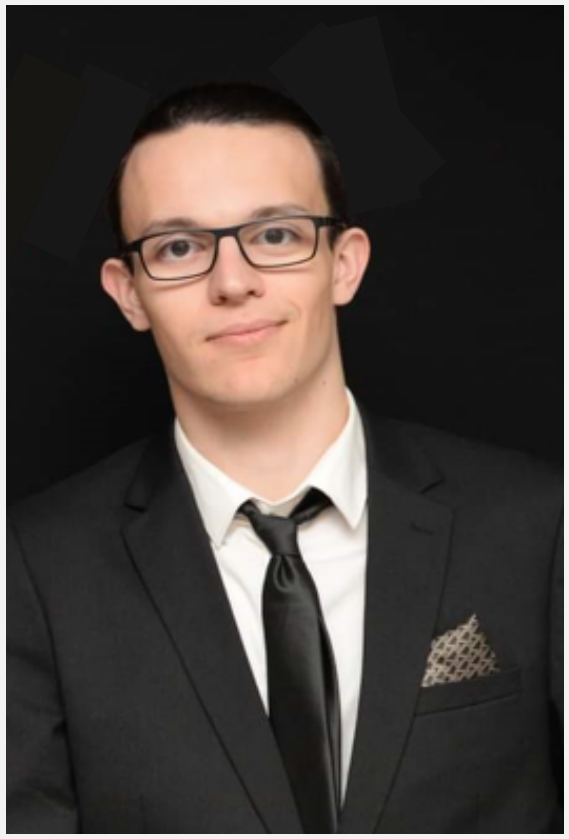

Qualifications

2020 - to date	**Chemical Engineering Degree** [*equivalent to Master Level*] National Polytechnic Institute of Chemical Engineering and Technology (INP-ENSIACET), Toulouse, France Graduation expected in 2023
2018 - 2020	**Preparatory Classes for Entrance into National Engineering Schools** Lycée Claude Fauriel, Saint-Etienne, France
2015 - 2018	**Baccalauréat Scientifique** [*Equivalent to High School Diploma in Sciences, with honors*] Lycée Claude Fauriel – Saint-Etienne (42)

Toulouse-INP ENSIACET at a glance ….

- Toulouse-INP ENSIACET is one of the largest engineering school in the fields of matter transformation and energy.
- Top 400 best schools in the Shanghai ranking for Environmental Science and Engineering (2021)
- 19/134 of the national engineering school ranking
- 300 students graduate per year
- 200 publications in international journals per year

- The University presents 5 majors:
  - Chemistry
  - Materials
  - Chemical Engineering
  - Process Engineering
  - Industrial Engineering

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TOULOUSE, THE PINK CITY

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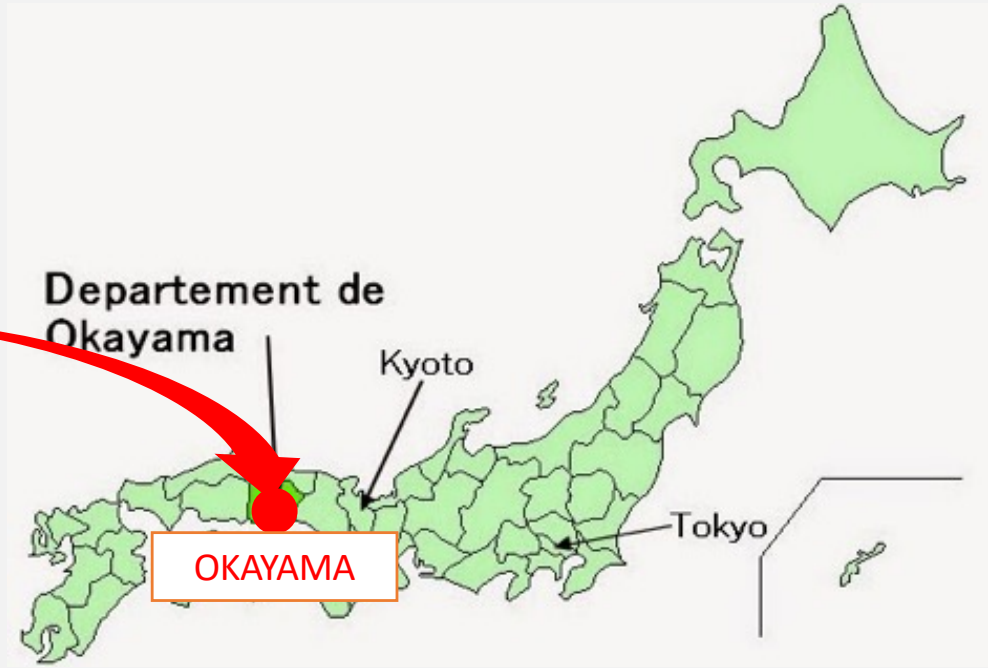
Why Japan ?

To experience the culture I look up to since my early years.



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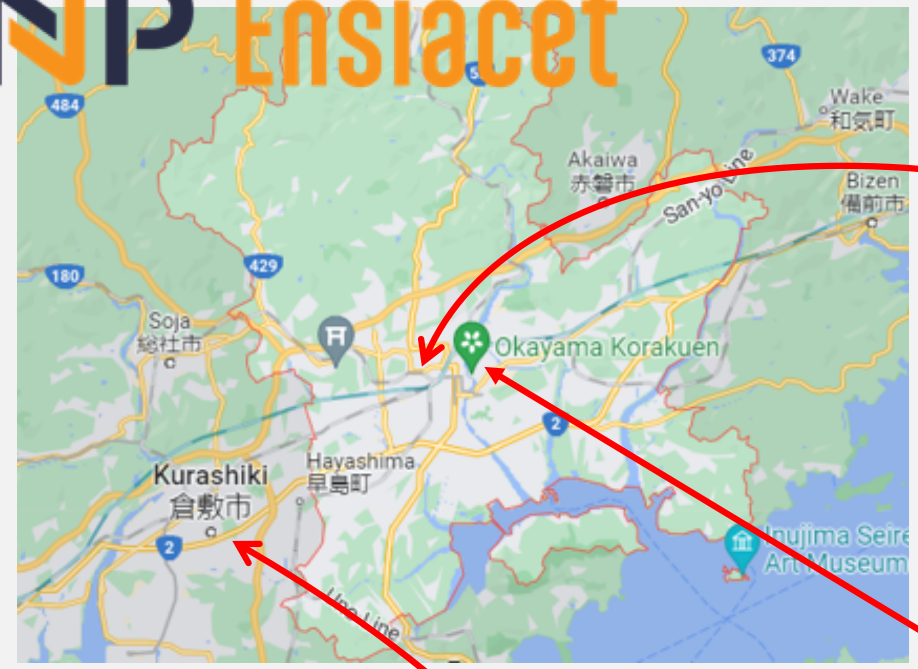
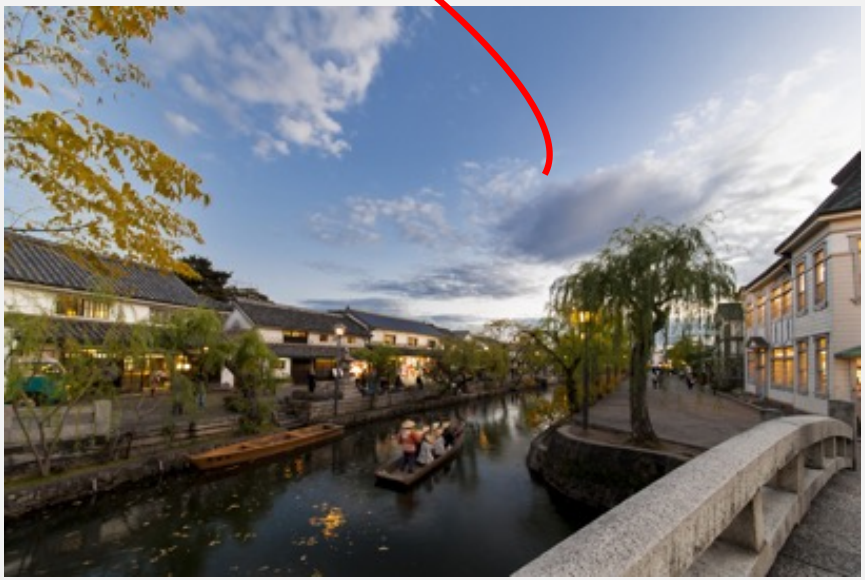


Okayama Castle



Kurashiki, Bikan Historical Quarter

Okayama Korakuen Garden

Okayama University in a few numbers

- 13 000 students, 1700 Faculty members
- 19th university in Japan ranking 2022
  Source: https://www.universityguru.com/fr/universites--japon
- 5 mains domains of research, with for instance Chemistry, Materials Science and Medical

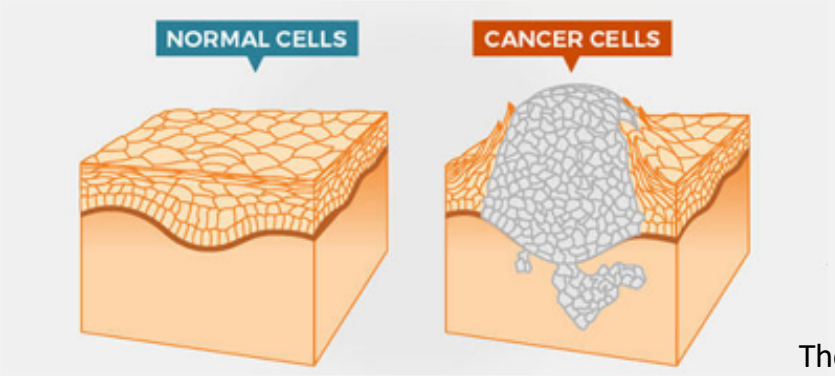
Context - I

- **In Cancer**, body cells **grow uncontrollably** and may spread to the entire body including tissues.
- Most common cancers are breast and lung cancers.
- Cancer in the world ➔ **10 million deaths in 2020**. It is currently impossible to cure advanced stages.
- A highly studied alternative: early stage detection to initiate efficient treatments.

Main issue: Find efficient, fast, non invasive, accessible and cheap method of detection to replace the mammography based current method

Most of the developed countries in Europe and America use mammograms. Downside: accessibility, malignancy and false-positive results caused by ionizing radiation.

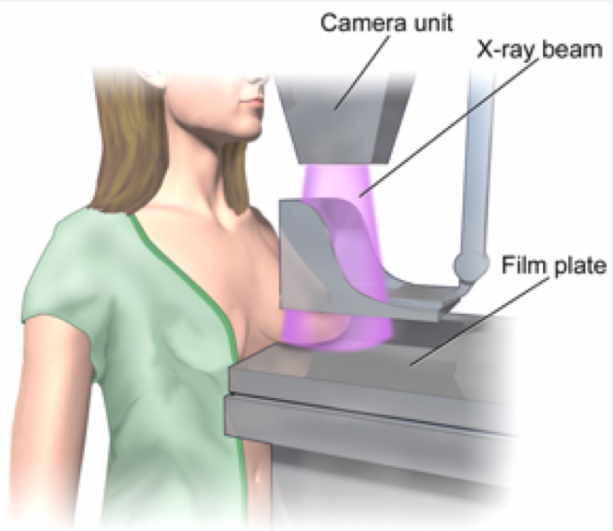
New device in India -> Thermography as an upcoming solution ?



Comparison between a normal cell and a cancer cell, source: https://www.cancer.gov/about-cancer/understanding/what-is-cancer

Thermogram by Indian entrepreneur Geetha Manjunath, source: https://www.lemelson.org/if-we-can-detect-it-early-we-can-stop-it/

Mammography illustration, source: https://undergradimaging.pressbooks.com/chapter/mammography/
https://undergradimaging.pressbooks.com/chapter/mammography/

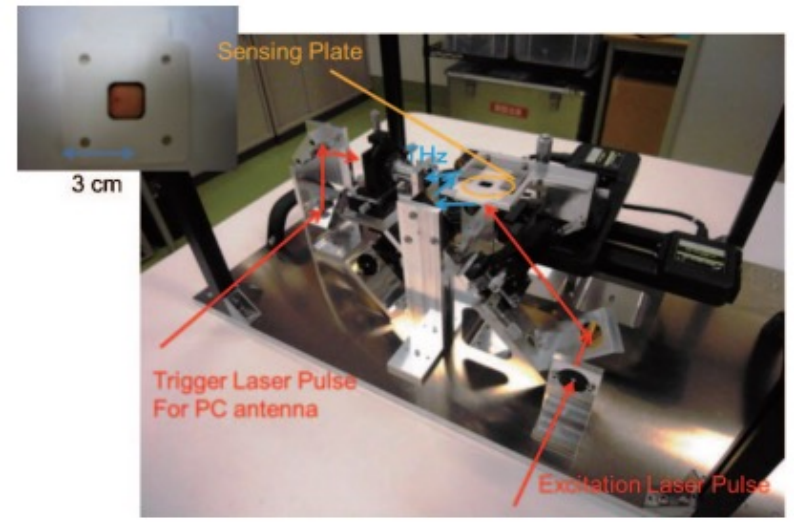


Mammogram

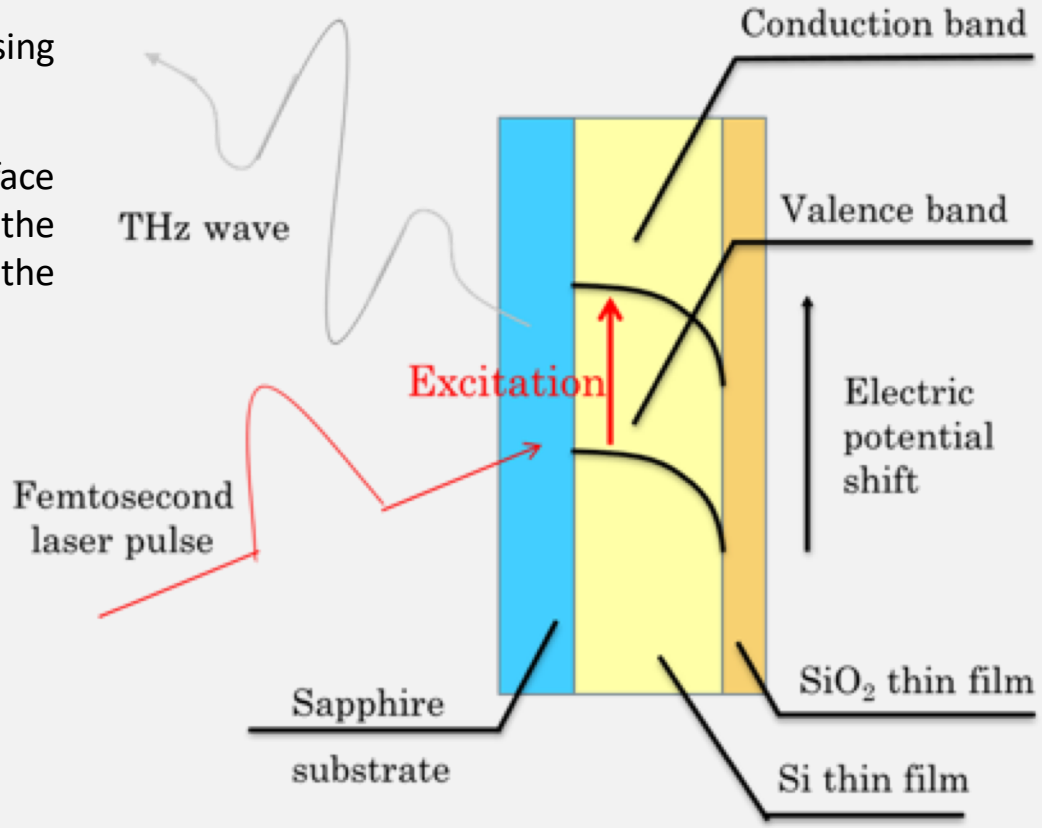
Context - II

Pr. T. Kiwa's research team started in 2007 to develop a microscope **for mapping chemical potentials of chemical reactions** in a gaseous atmosphere and aqueous solutions [2]: a **TeraHertz Chemical Microscope (TCM).** It is an ultra-fast method of detection.

- When the femto-second laser pulse hits the sensing plate by the backside, the THz pulse is generated.
- If the chemical reaction located on the insulator surface proceeds and the chemical potential at the surface of the insulator layer shifts, it will lead to changes in the magnitude of the local field changes.



Prototype TCM system [2]



Schematic diagram of the THz wave emission

Context – III:

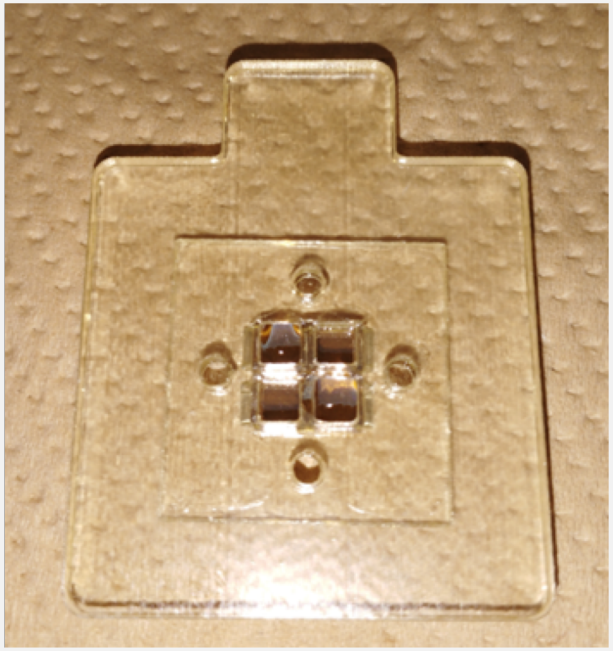
Positioning my internship

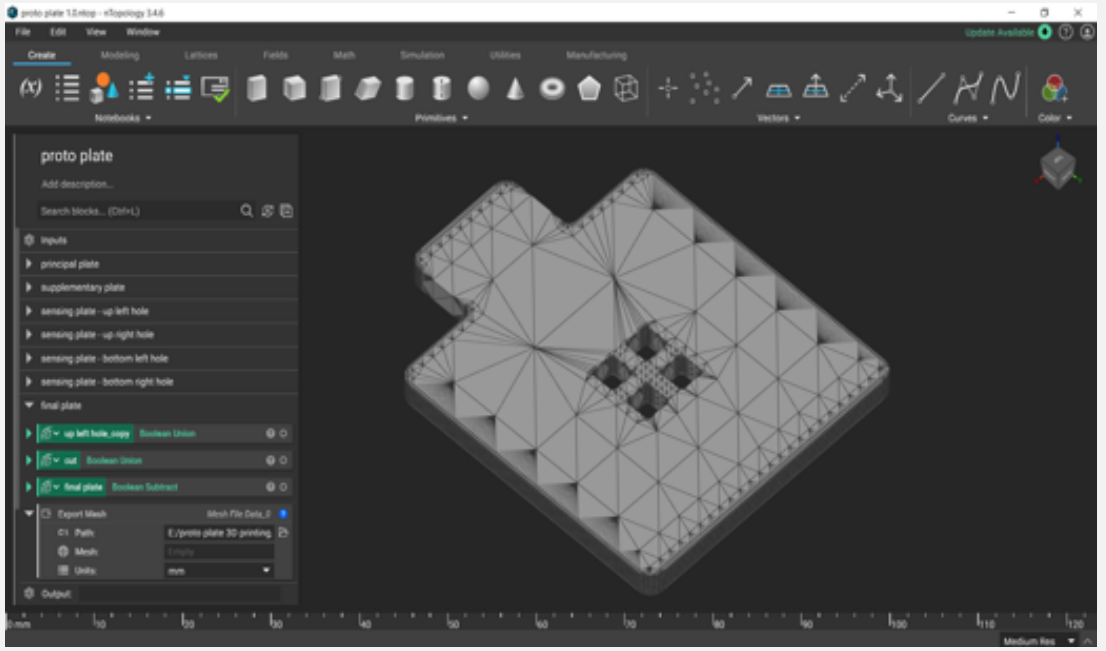
Further develop the TCM for medical applications – Specific focus on breast cancer early detection --
The objective is to 3D manufacturing a new holder to improve the TCM analysis.

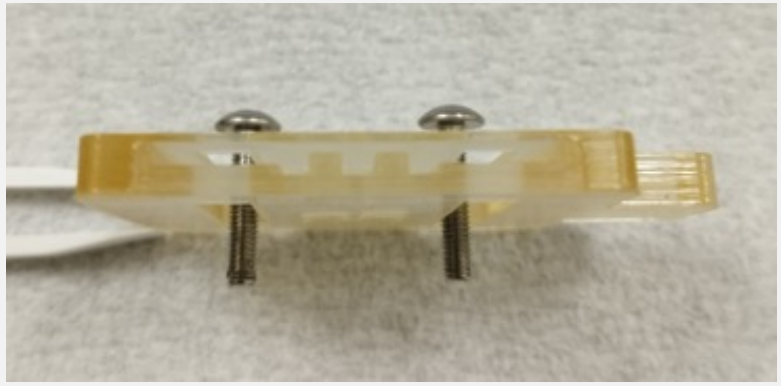
- **Main objectives of the internship:**
  1. Understand the TCM system basic principles
  2. Design an optimized holder for the analysis without the current problems.

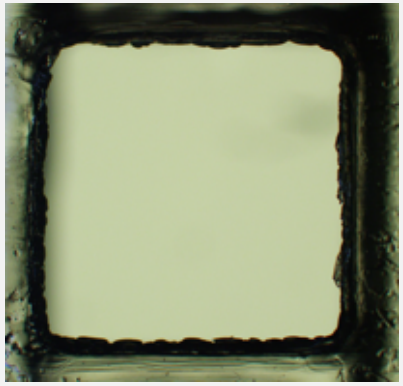
- **Methods:**
  1. Using the current plate as a reference
  2. Using 3D Modelization and Printing to conceive the new holder
  3. Trial and Error

Design of an optimized plate

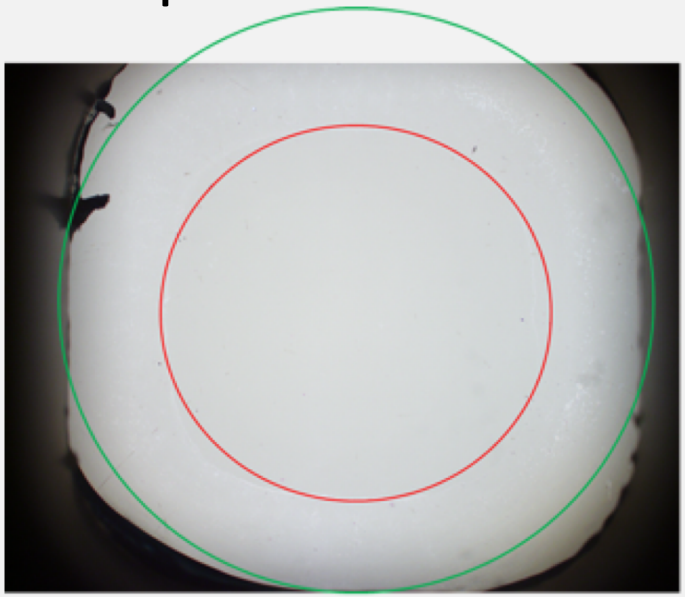






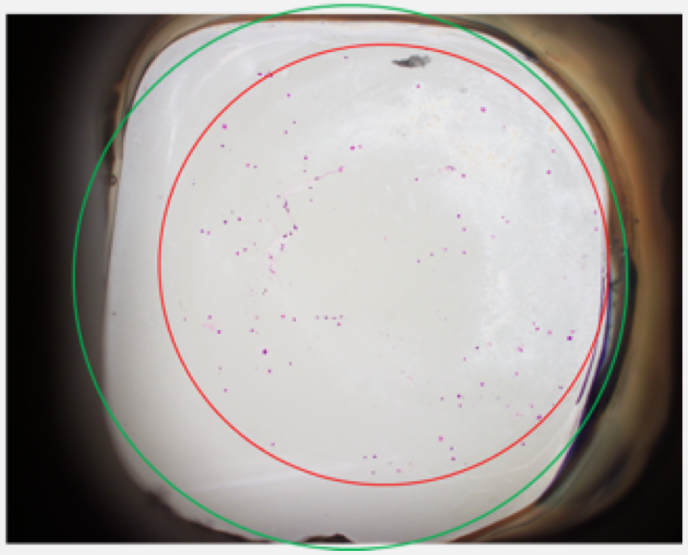


The backlash of using manicure, a marge for improvement



Effective surface 56.2 %

$\Rightarrow up\ to\ a\ 43.8\ \%$ surface gain



Effective surface 66.8 %

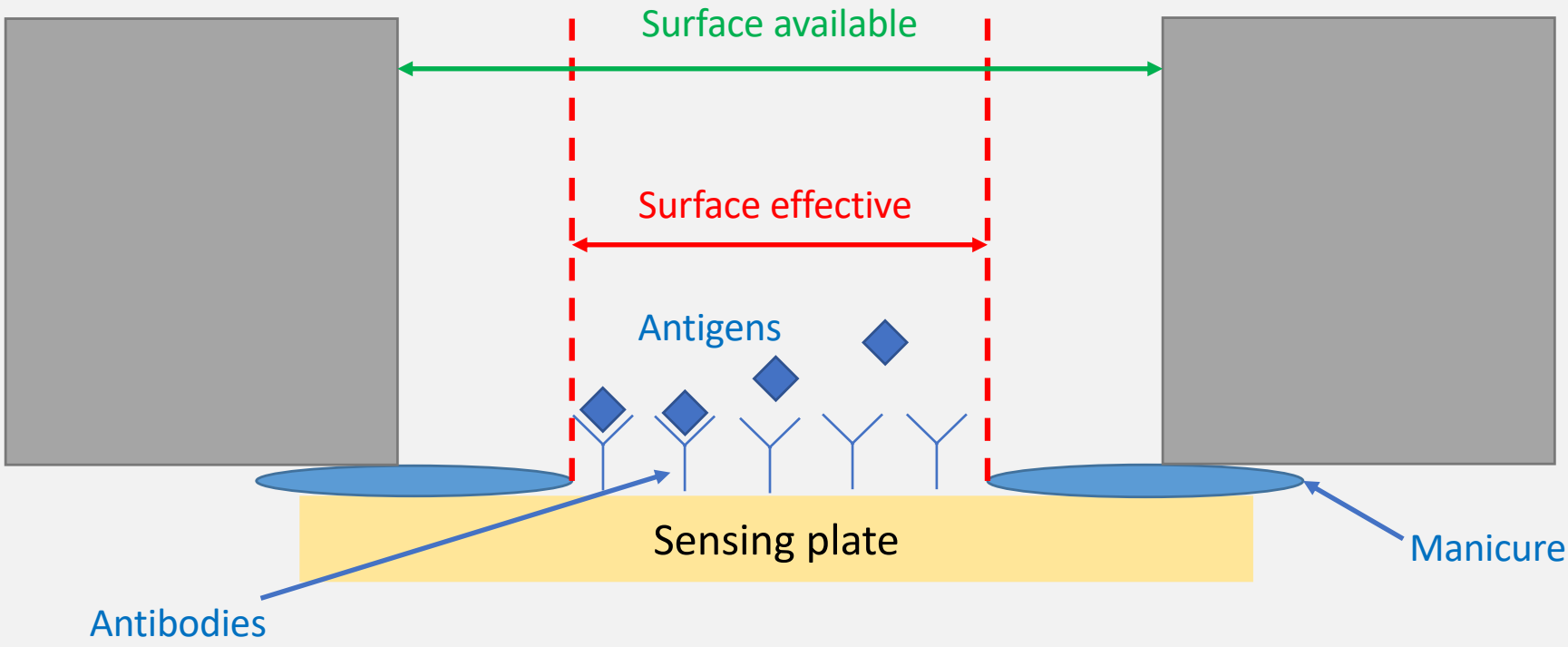
$\Rightarrow up\ to\ a\ 33.2\ \%$ surface gain

First approximation:

- The green circle is consider equivalent to the well surface
- The red circle is consider equivalent to the effective surface limited by manicure

Highlighting of the restriction of the available field on the sensing plate by manicure

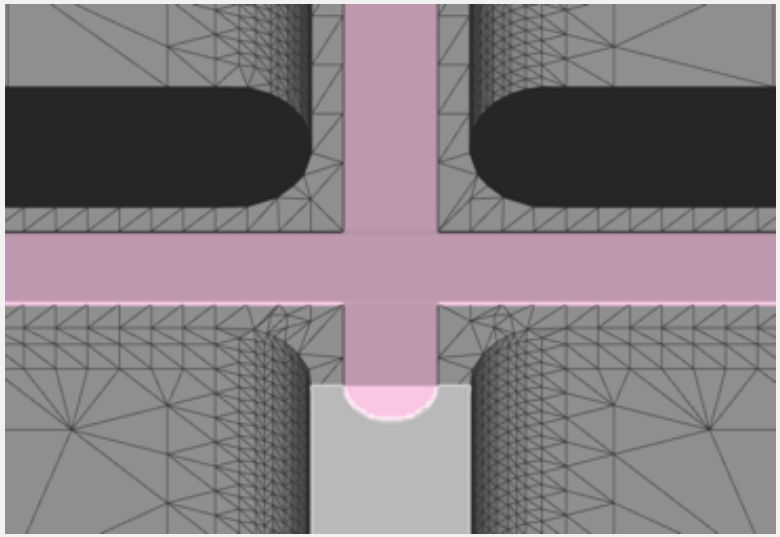
A quick explanation of the problem



Schematic diagram of the manicure spreading matter

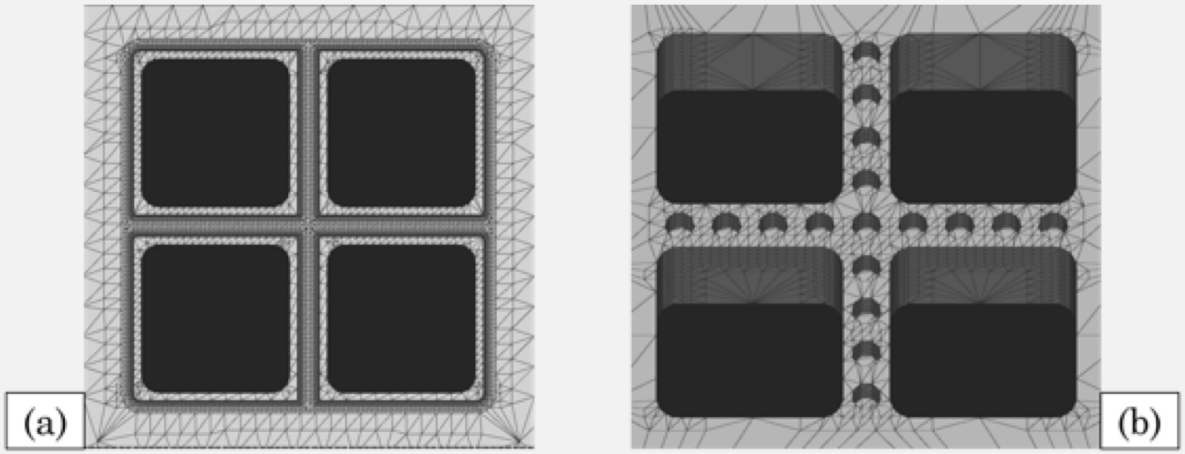
2 possible solutions, keeping or removing manicure

- The first solution thought was to change the shape of the boundary surface between the sensing plate and plate.
- The hole model was quickly abandoned due to the disruption of continuity between each of the wells.



Cross-section of the trenches of the 3D model with manicure

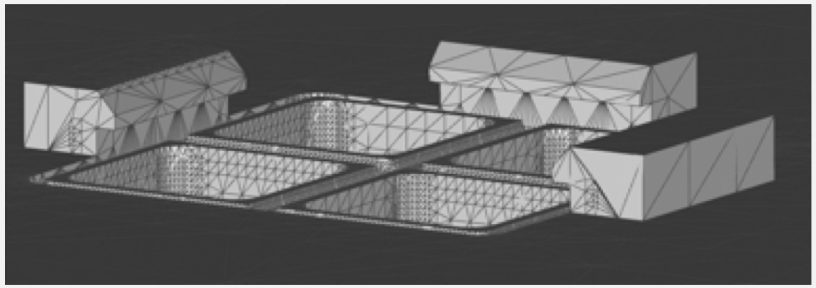
(a) Plate with trenches 3D model and (b) plate with holes 3D model

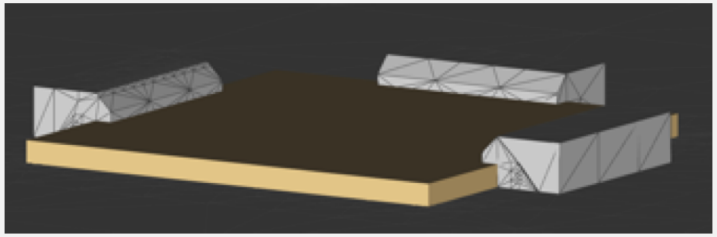


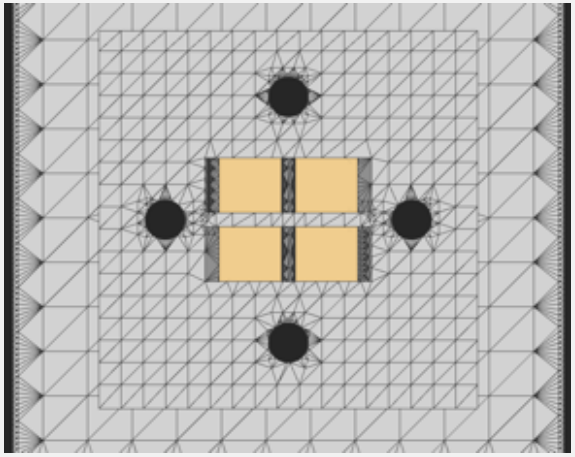
- The theoretical advantage of the trench model was that it should have making it a plane surface.
- However, the plate actually lacked efficiency as it was impossible to apply manicure due to its high viscosity and the small space allowed by the trench (0.3 mm of radius).

Is it possible to proceed without manicure ?

- One of the first prototype designed was the hook model. The sensing plate would be slid in.
- Yet, no manicure = no insulator. As such, the holder cannot contain the solution by itself.
- As a matter of fact, not enough pressure is applied in the center to prevent leaks from one well to another.

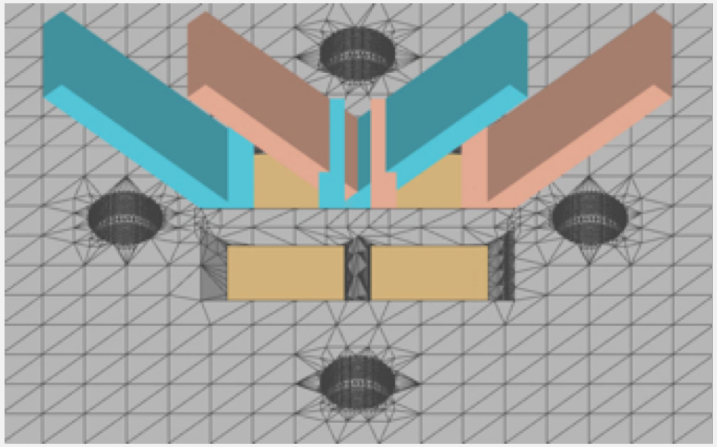




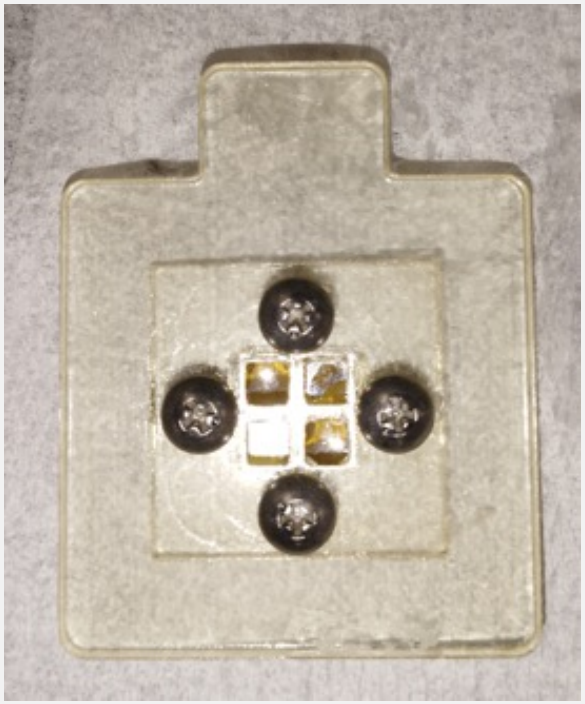


Above view of the screw plate 3D model with the sensing plate

- By using a supplementary plate to lock the sensing plate and screws, pressure could be applied on the entire sensing plate.
- The shape was cut in a 45° angle to not disrupt the laser trajectory.



Top view of the supplementary plate with the laser trajectory reflecting on the sensing plate



Less than 1 min later

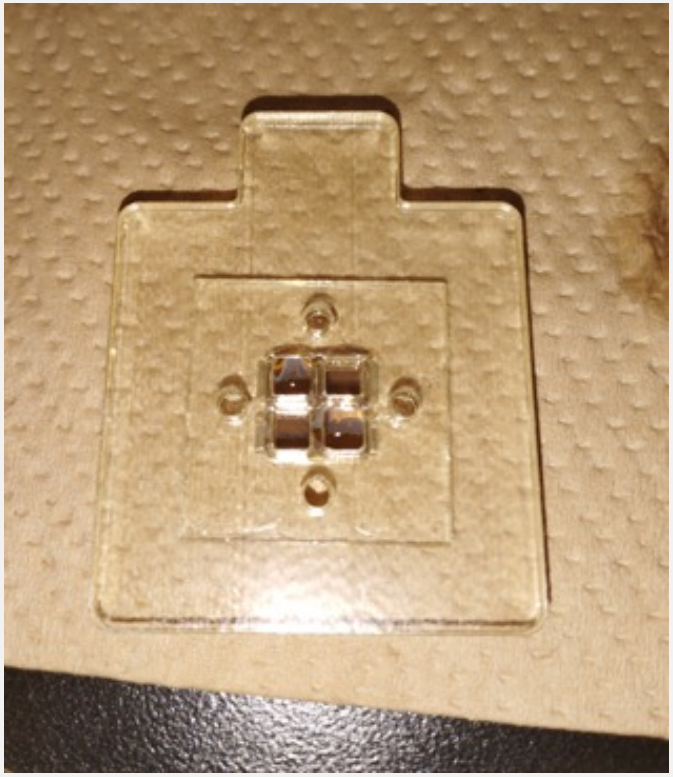
Figure 25 – Waterproofness test of the 3D printed screw plates

- M3 screw were used to attached the plates together.
- The volume available for each well is 42 µL.
- After waiting, a small leak is visible. As a matter of fact, without manicure it is difficult to control the pressure applied to the center.

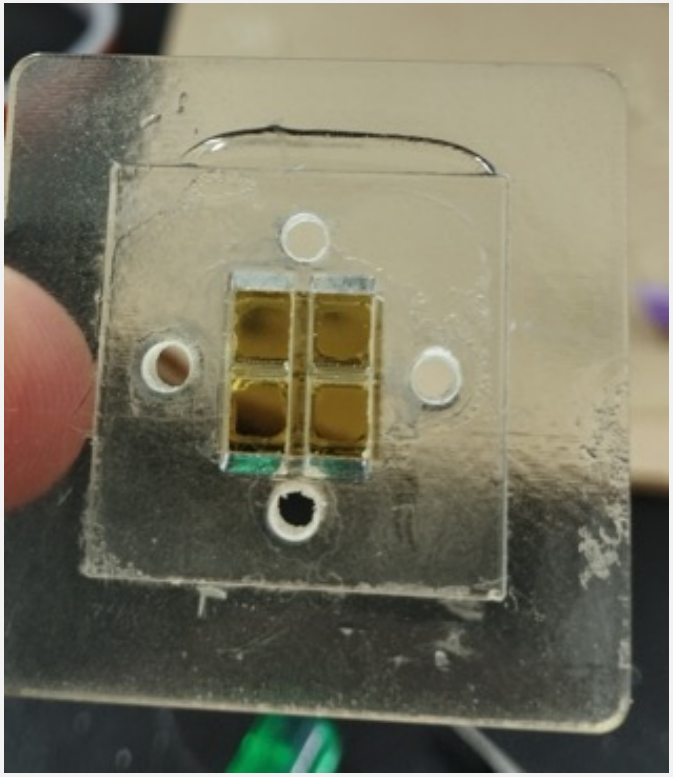
⇓

To conclude, it is not possible to proceed without manicure, so there is a need to find an alternative to manicure.

The use of a silicone spray, the solution to the leaking problem

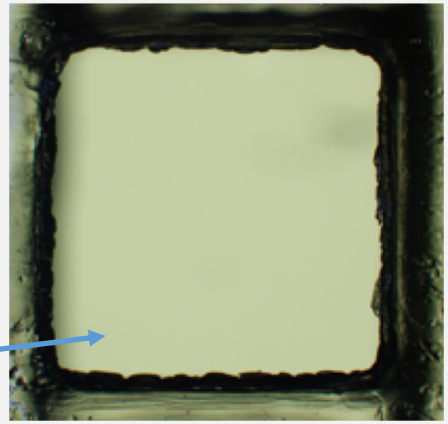


Photos of the functionable plates sprayed with silicone



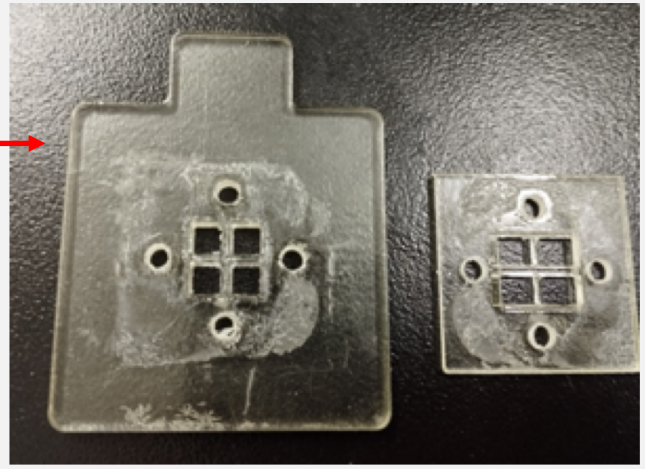
Conclusions to the properties observed :

- Advantages:
  - The waterproofness is sufficient (volume max = 40 µL).
  - The problem of the manicure expansion is prevented and there is a surface gain up to 40% for some well.



Photograph of the improved plate

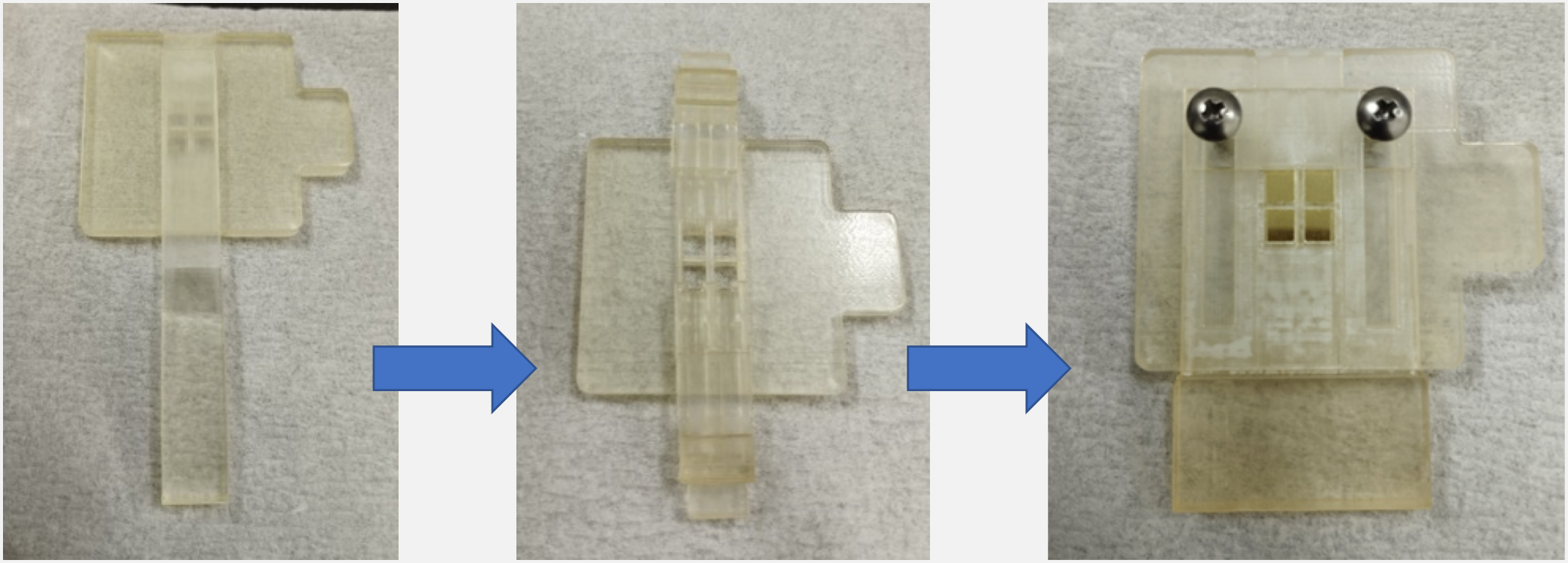
- Drawbacks:
  - Using 3D printing, the plate can only be used once as the gluing effect of the silicone layer is too potent.
  - Lack of efficiency due to a 3 days preparation.



Showcase of the damage on the surface of both plate after using the silicone spray

⇒ Need for a more efficient and reusable plate.

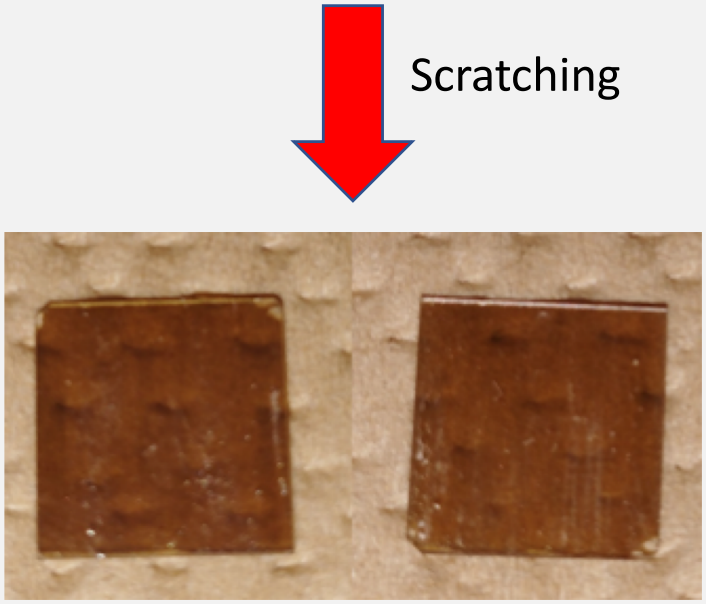
The potential of a plate with a sliding overture



Evolution of the sliding holder prototypes

Conclusions to the properties observed :

- Advantages:
  - The plate is very practical.
  - The plate is reusable and does not need a lot of time to be prepared.
- Drawbacks:
  - The leaking problem is present since no insulator was used.
  - There is a scratching of the silicone surface due to the a lack of space for letting inside the sensing plate which could remove the antibodies and the cancer cells immobilized.



Display of the scratching on the sensing plate

⇒ Need for an insulator and a way to reduce the scratching.

Conclusion

- The objective was to improve the TCM analysis by designing a plate without the manicure spreading and the cell distribution matters.
- To solve the manicure spreading problem, the goal was to design a plate which would take into account four criteria:
  - No leaking
  - A reusable plate
  - A movable sliding sensing plate
  - A reproducibility of the experiments
- Only the plate which used silicon spray to fix the supplementary plate is waterproof and able to solve the manicure spreading matter. To make it reusable, using ceramic as a material could be an option.
- Another option could be to try to improve the last sliding plate, as it possesses a lot of potential since it does not need three days to be operational.

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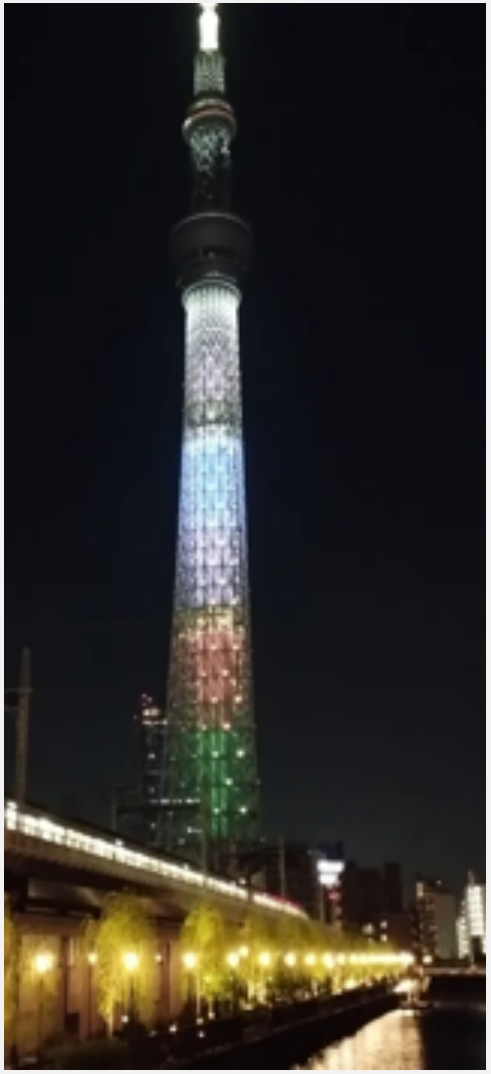
日本での
この4ヶ月間

ありがとうございました！



Acknowledgments

This research Internship would not have been possible without:

A - Okayama University for accepting my Internship application

B - Pr. KIWA's group for kindly and very professionally supervising my Internship and giving me the opportunity to learn about the use of THz wave to detect cancer cells by recording the potential shift. The team also gave me opportunities to use the TCM with my coworkers and better understand the process of this system with the use of as a control software .

C - Pr. H. INOUE from Okayama Hospital for making me discover the bacteriologic section as well as all the equipment needed to prepare the cancer cell preparation. I also want to thank him for his interest, his support and his ideas which help me during the conception of the plate.

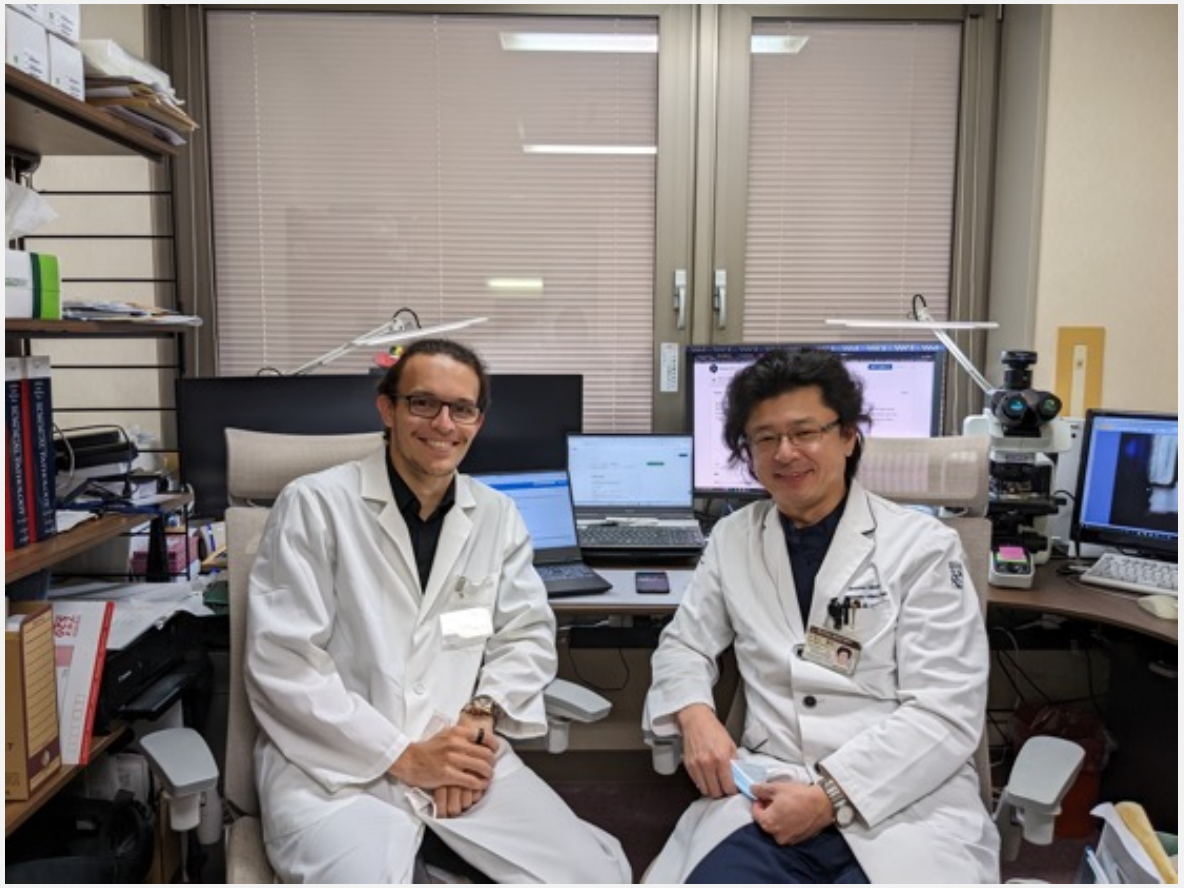
D - Pr. K. SAKAI for is constant availability, technical support and help, as well as my co-worker for welcoming me and taking care of me.

E - The URA division of Okayama University:
URA's and in particular Dr. B. CHENEVIER – senior Researcher, on leave from CNRS – who proposed the Internship after discussion with Pr. KIWA. He also guided me through the arcane of Okayama University administration and advised me for my oral presentations.

Yayoi KUSAMA yellow pumpkin on Naoshima Island
In the Okayama bay

URA stands for University Research Administrator

Any questions ?



Prof H. Inoue and Tanguy Dusson – Septembre 8 - 2022

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- [2] Toshihiko Kiwa, "Unique terahertz chemical microscope for mapping chemical reactions", Okayama University e-Bulletin, Vol.4, September 2013

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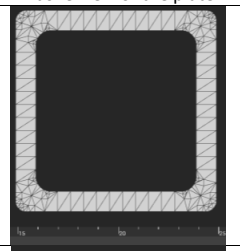
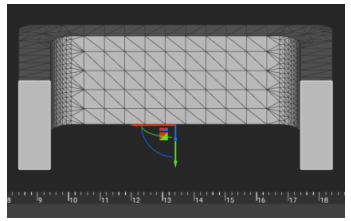
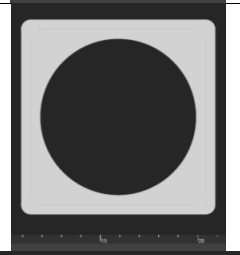
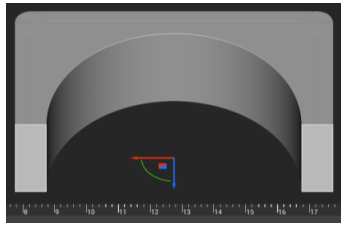
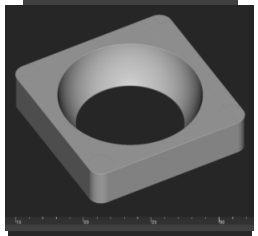
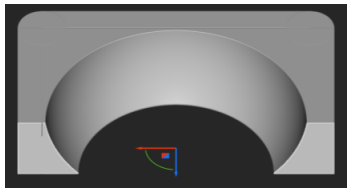
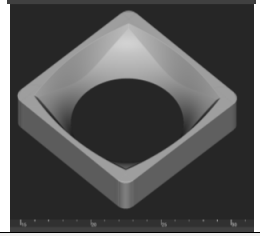
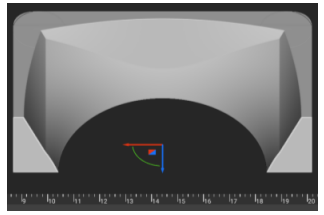
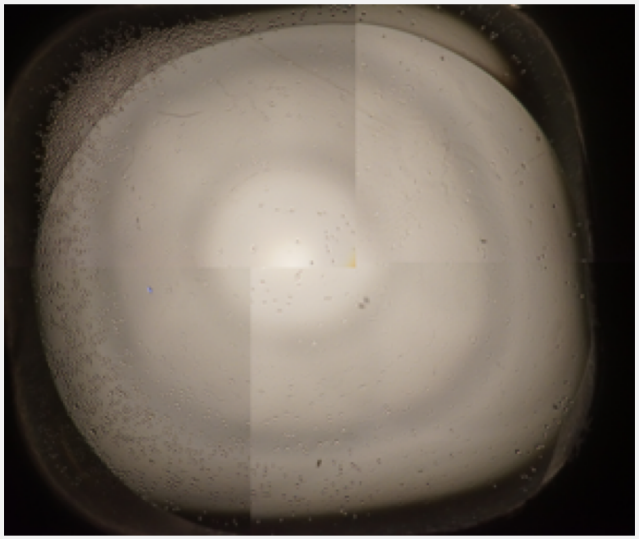
Annexe -The cell distribution problem

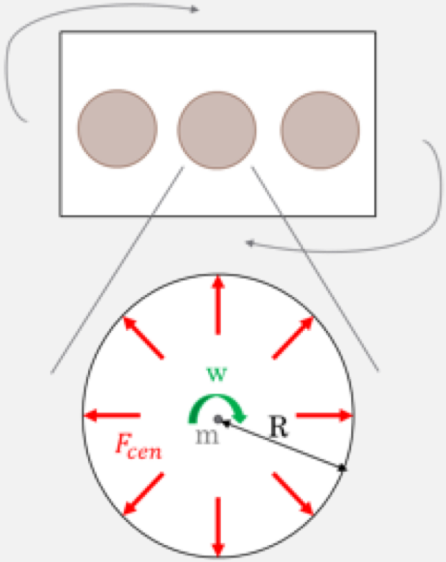
- Most of the cells tend to agglomerate on the side of the well, leaving the center near empty.
- Most likely due to the centrifugal force, the equation obtained by the kinematics study highlights the influence of the rotation speed.
- New plates were tested with different edges to see if the shape of the well could affect the cell distribution.
- Since the observations made seemed to align together, it was concluded that there were not any major differences between the different types of edges.

Display of the four well edge types

Type of Edge	Above view of the plate	Cross-section view of the plate
Rectangular		
Circular		
Spherical progressive		
Circular progressive		



Photograph of one dispatchment observed



$$F_{cen} = m \,.\, w^2 \,.\, R$$

Schematic diagram and hypothesis of the problem