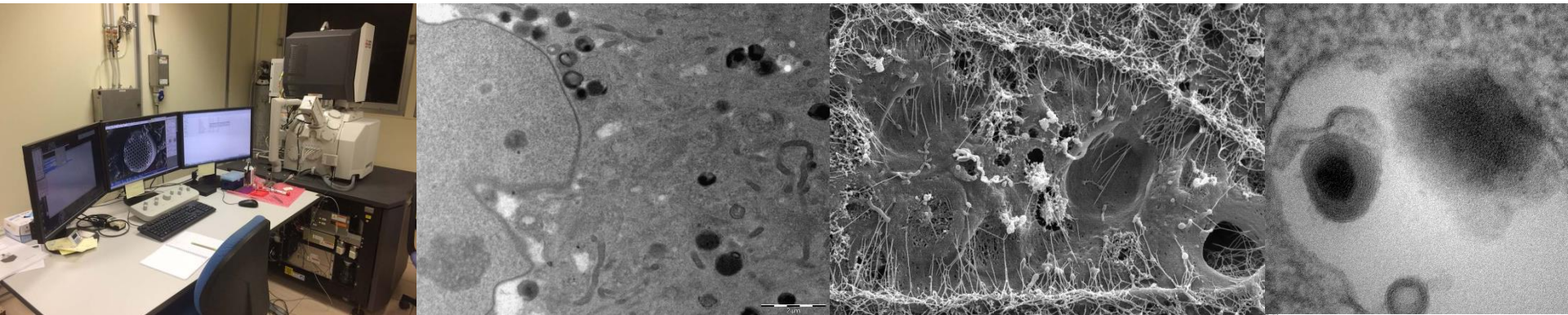


review *(2020-2021)*

Imaging facilities



We deal about :

- Review :
 - Team
 - Uses
 - Training
- Technical overview:
 - CAO / Impression 3D : realizations
 - New light sheet : Blaze
 - Oil choice
- Survey 2021
 - Key points
 - Conclusions
- Electron Microscopy
- Open Discussion

TEAM

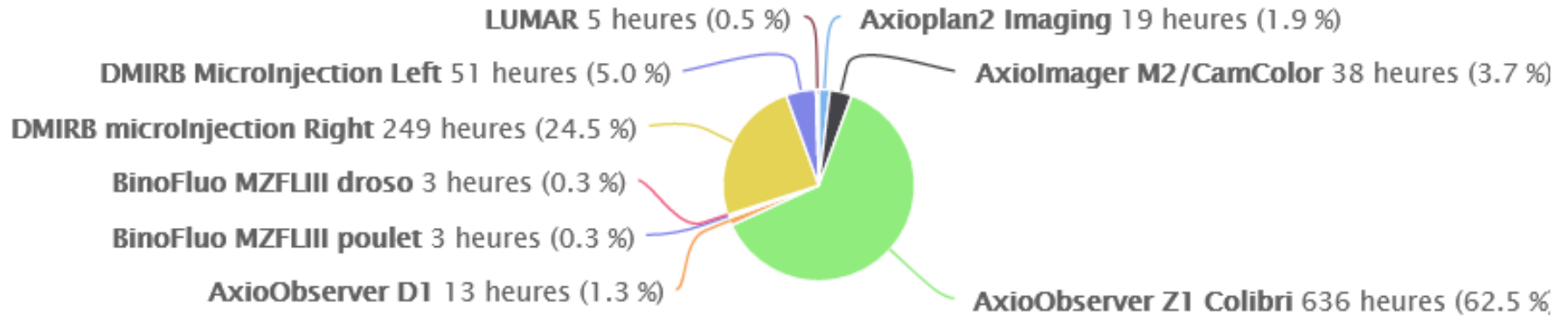
- New collaborators :
 - Daniel Sapede: PhD, 2 years experience on imaging facility, 9 years experience as field service engineer (Zeiss).
 - Sotirios Papadiamantis : engineer E-management deployment, computer scientist
- New students master2 : coming soon
 - Youssef Ayachi : omero and metrology pipeline development
 - Gabriel Bon : microscope augmentation by deep learning



- Resource management software to replace Microbook
- Microscope bookings, maintenance, training
- Detailed statistics of resource use per group and resource
- Automatic billing
- Scientific project management
- Client-server software
- Microscopy image and associated metadata management
- Image data processing and analysis with various tools (ex. ImageJ, Tapas)
- Dataset visualization and publishing

Use of microscopes : Elsa

Nombre d'heures par équipement



- 2530h LSM880
 - 610h Ropper Spinning Disk
 - 1570h LSM780
 - 481h LSM510 (Zen-NanoIndentation)
 - 228h LSM510MetaUV
- 5419h
- 566h STED

- 123h 7MP
 - 341h 510NLO
- 467h ApoZ1
 - 920h ApoM2
 - 682h ObserverZ1Apo2
- 2069h

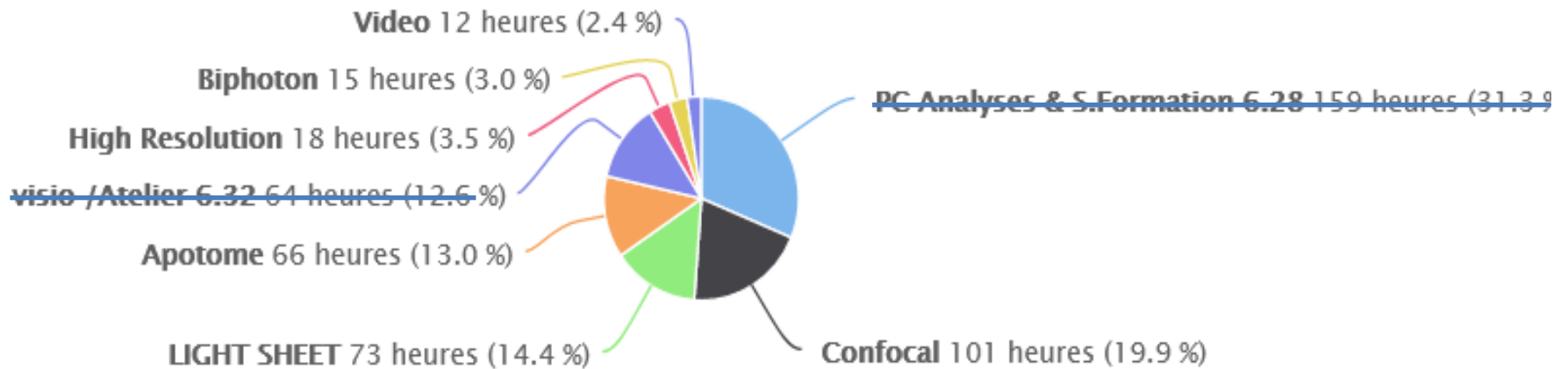
Légende:

- IBDMBP1
- IBDM TPR2

- Réserver les systèmes pour justifier de leur utilisation en vue leur remplacement (facturation):
BP1 & Video microscopes
- Toujours une forte demande pour le confocal
- **832h dé-réservées et non reprises**
- **193 utilisateurs de la plateforme**

Trainings

Per System



- 2019 = 109 Formations
 - 2020 = 47
 - 2021 = 44
- } Covid year

Review of breakdowns

- **AxiolmagerM2 (6.19)** : changement de la pile 3V de la carte Main Bord lors de la révision annuelle (*sous contrat* 😊).
- **ApoM2** : New MCU28 et Joystick. (*recup!* 😊) + PB écran TFT (*sous contrat* 😊).
- **AxioObserverZ1APO2** : PB port com “platine” changement ordi (*recup* 😊!)
+ Répar Obj20x (2020) + Répar Obj 20x et 63x (2021) (*coût: 3x 3500€ !* 😞)
- **LSM510Meta** : Chgmt PC et Disque dur Data (*recup dans notre caverne d’Alibaba* 😊)
- **510NanoIndenteur** : Chgmt PC (*recup* 😊!) + répar NanoIndenteur (*sous contrat* 😊).
- **LSM780** : Chngmt Alim Argon + changement PC (*sous contrat* 😊! Sinon : 30k€) + *Détecteur Spectral*
En cours de réparation (sous contrat 😊! Sinon 80k€)
- **LSM 880** : Chgmt laser 561nm (*sous contrat* 😊! Sinon 20k€) chgmt laser Argon + AOTF (*sous contrat* 😊! Sinon 15k€)
- **Spinning Disk** : Réparation du shutter manuel du micro (*home made* 😊!)
- **STED** : Chgmt détecteur Hybride “gating” (*sous contrat* 😊).
- **LIGHTSHEET** : chgmt refroidisseur (*sous contrat* 😊 sinon 3k€)).
- **LSM510NLO** : Chgmt Alim électronique + Hautes tension PMTs internes (*recup* 😊!)
+ Détecteur GaAsp AlphaNov : **OUT !** Coupure élect...(à suivre !...)

❖ Balance sheet between repair cost estimation and sav cost : 148 k€ - 120 k€ = 28k€

Lest 2 years evolutions

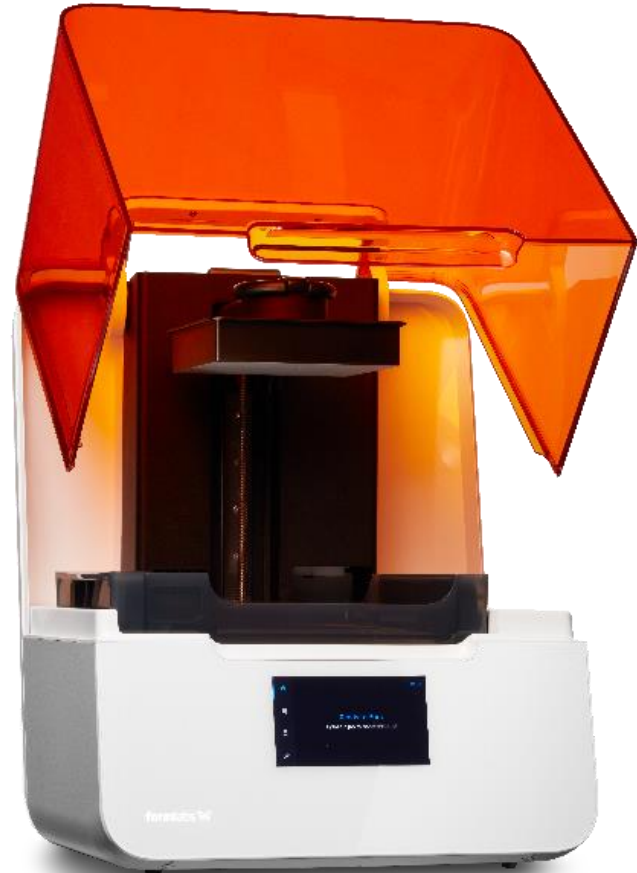
- AxioObserverZ1Colibri & APO2 / AxioImagerAPO Z1&M2: Caméra hamamatsu Flash 4LT
(Commission achat 2019 + financement institut imagerie) : 32 k€
- AxioObserverZ1APO2 : Win10 + Zen 2.3 (upgrade gratuit)
- AxioObserverZ1Colibri : Win10 + Zen 2.6 (upgrade gratuit)
- LSM880: Universal incubation chamber OKOlabs: (Commission achat 2021 : 15k€+ financement Mehdi : 5k€)

Technicals overview : Brice

3D printing



Dépôt de matière fondue



Stéréolithographie

Elaboration d'un projet

1. Définition du cahier des charges

- Conditions environnementales d'utilisation
- Conditions d'utilisation
- Morphologie
- Dimensions et précision
- Pièce unitaire ou série

Matériau = Technologie
d'impression 3D

2. Représentation 3D du projet (CAO)

3. Echanges autour du modèle 3D

4. Impression 3D d'un prototype

5. Tests de fonctionnement

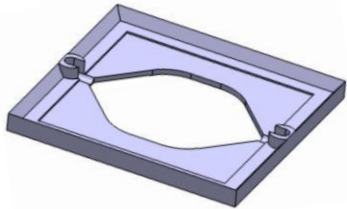
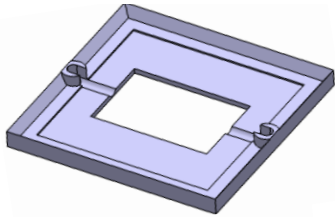
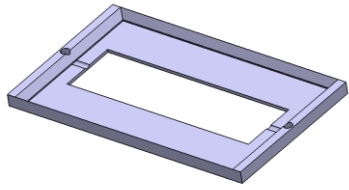
Modifications
Evolutions

```
graph TD; A[Modifications Evolutions] --> B[2. Représentation 3D du projet (CAO)]; A --> E[5. Tests de fonctionnement];
```

Impression 3D (FDM)

Equipe Mann

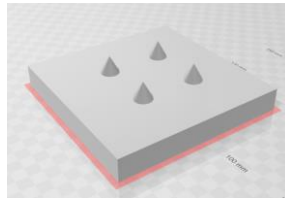
Elaboration: 1 mois 1/2



Hauteur 2 mm
côtés 27 mm

Eqp Mann

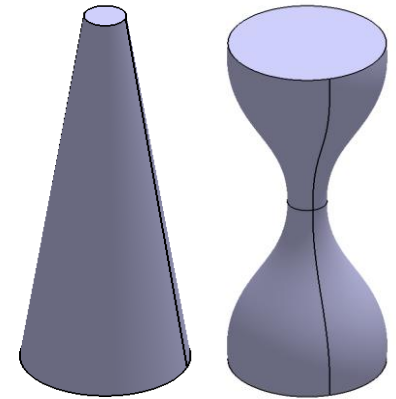
Support pour l'Ultramicroscope
pour clipser les pancréas



Hauteur 2 mm, côtés 150 x 150 mm
Cônes base: 0,8mm de diamètre

Equipe Durbec

Elaboration 5 à 6 heures



Hauteur 120 mm
Diamètre 40 mm

LSM 510

Elaboration 9 mois
Système de chauffage
pour Petri de 35 mm



Perspectives

Micro fluidique

Limitation dans la taille et la forme des canaux

Formation avril 2022

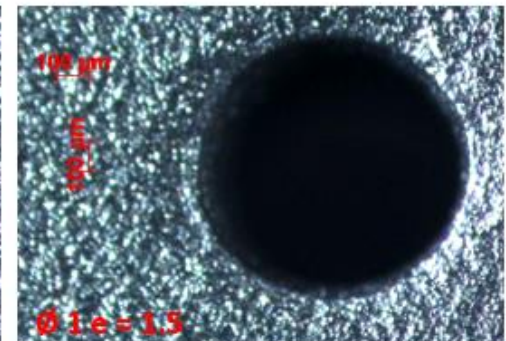
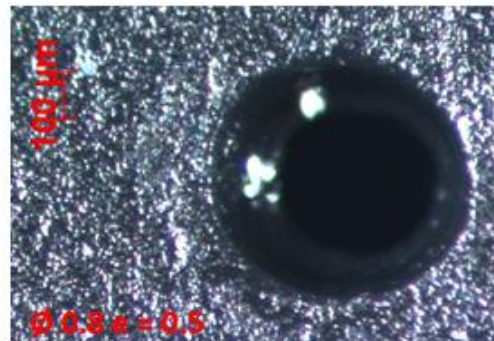
Investissement dans une technologie plus performante en micro fluidique, en fonction des besoins?

Projet Equipe Le Bivic



Tests de capacités d'impression en fonction du matériau

		Diamètres théoriques des trous (mm)															
		0.3		0.4		0.5		0.6		0.7		0.8		0.9		1	
		X	Y	X	Y	X	Y	X	Y	X	Y	X	Y	X	Y	X	Y
Epaisseur Des Trous (mm)	0.25											0.58	0.55	0.71	0.67	0.83	0.79
	0.5											0.55	0.53	0.67	0.67	0.85	0.82
	0.75											0.58	0.56	0.67	0.67	0.79	0.75
	1											0.59	0.57	0.67	0.75	0.82	0.77
	1.25											0.52	0.55	0.67	0.66	0.84	0.82
	1.5											0.57	0.58	0.73	0.73	0.87	0.85



Lightsheet : Elsa

Ultramicroscope (BLAZE)

Thanks to Fanny and Harold



Observation de gros échantillons
plusieurs cm



Cuve 450mL



≠ supports Surface
plane de 0,5/1,5cm ;
0,5/3cm ; 0,5/5cm

Objectifs

1x/0,1
4x/0,35
12x0,56

Lasers

488nm
561nm
640nm
785nm

Caméra sCMOS

2560x2160 pxl
Pxl: 6,5x6,5µm
Sensor size:
16,6x14mm²

Transparisations

autorisées

Cubic n=1,49

Eci n=1,5595

Salle 6.16 : PB Odeurs?

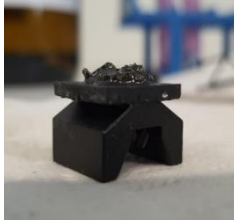
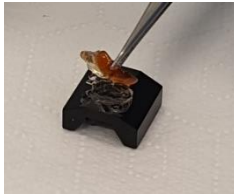
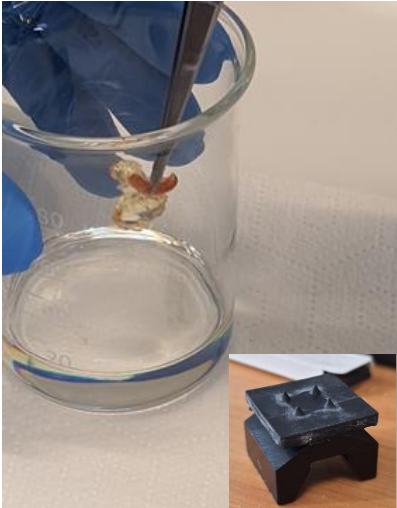
Pas de transparence en DBE, ni BBAB

Lightsheet

Ultramicroscope (BLAZE)

Thanks to Fanny and Harold

Eqp Mann - Angélique Puget
Pancréas de souris

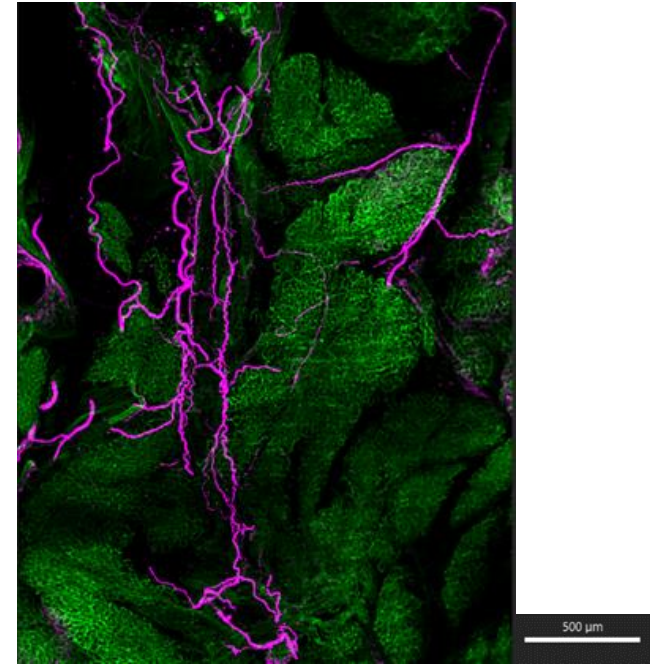


Observation du profil de
l'innervation sensorielle dans
le pancréas

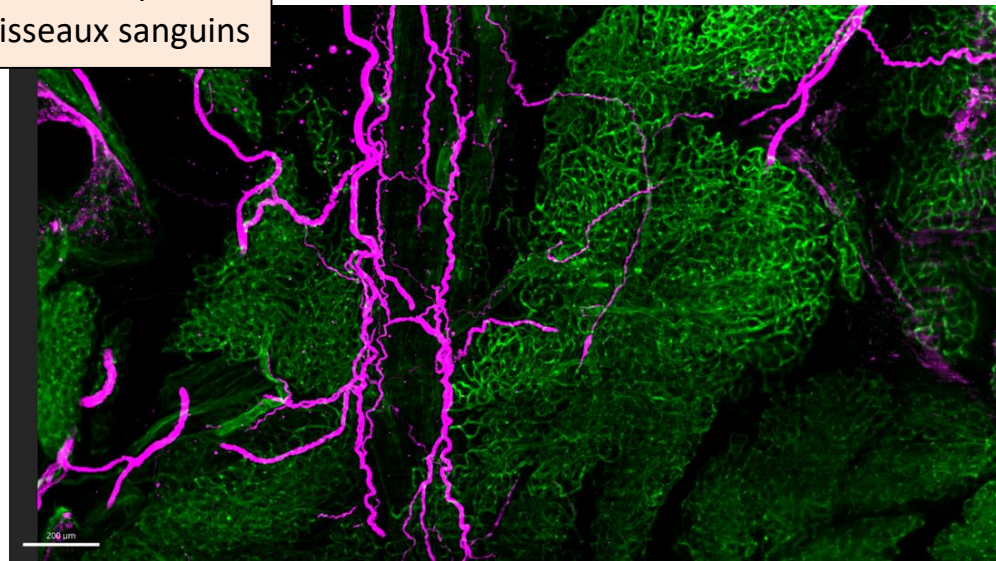
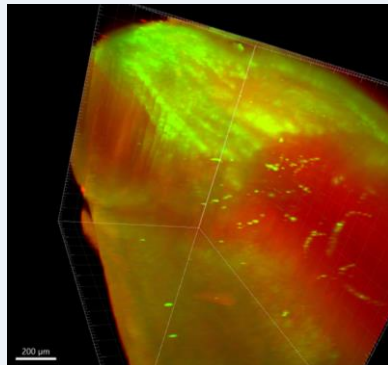
(Ici échantillon tumoral, ce
n'est pas la tumeur qui est
observée)

Obj4x zoom1x ; épaisseur
feuillet 4 μ m, sheet width 50%,
pas de 2 μ m.

Anticorps **NF200** pour marquer
les nerfs/fibres sensorielles et
Podocalyxine+MECA32 pour
marquer les vaisseaux sanguins



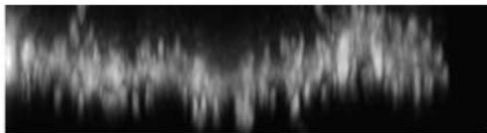
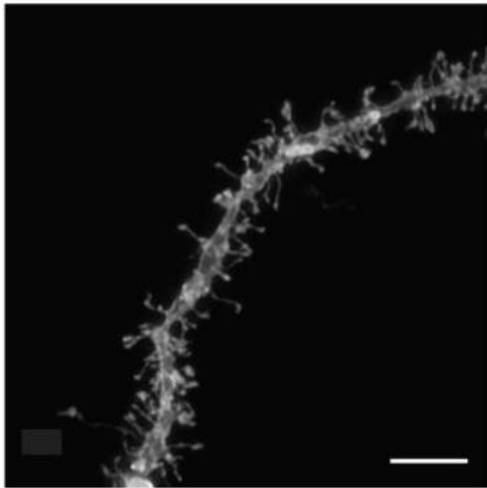
Déc 2021 : Premier essai :
Eqp Moqrich – Irene Marics
Moelle épinière de souris
Observation des nerfs
sensoriels



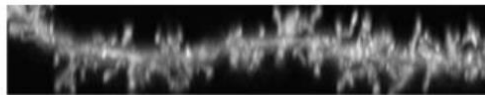
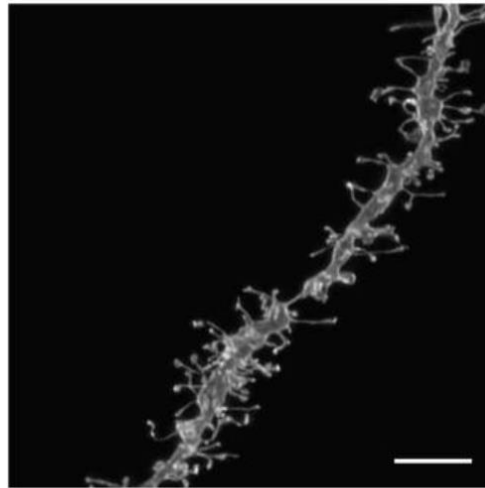
Oil immersion for incubation chamber : Daniel

- Ideal situation: same refractive index everywhere = objective, immersion, glass, sample.
- Reality: index of **sample media** and of **immersion** are difficult to control and **temperature dependant**
- Consequences of index mismatch: all kind of artefacts, mainly **z-axis deformation** (see below). Also strongly affect **chromatic correction** (think about it if you do colocalisation...)

$n = 1.470$



$n = 1.515$



Rat dendritic segments. Confocal Imaging. Scale bar is 50 μ m.

Brittni M. Peterson et al.

J Neurosci Methods. 2015 Mar 15; 0: 106–111.

Published online 2015 Jan 17. doi: [10.1016/j.jneumeth.2015.01.014](https://doi.org/10.1016/j.jneumeth.2015.01.014)

Open questions:

- Here 1,515 gives **better** results. So it seems easy: use the correct oil!

But which precise index would have provided the **best** results?

- Influence of temperature: We have oil for 23°C, 30°C, 37°C.

Are they the right call? What if you work at a different temperature?

- What if the sample media also provide artefacts? (thick samples)

Solution:

- To start with: pay attention to the oil you are using, and at which temperature. **Ask the facility for advices.**
- For those who need « the perfect image », we might test **sets of oils** or **home made mixes**, with small index variation, to find the perfect one for your condition.

2021 survey , key points : Cédric

What is your statut?

Réponse	Décompte	Pourcentage
PhD (AO01)	8	20.51%
Researcher (AO02)	18	46.15%
ITA (AO03)	9	23.08%
Master's trainee (AO04)	0	0.00%
Post-doc (AO05)	4	10.26%

Degree of satisfaction of the faciltie's instruments

Spinning disk

Réponse	Décompte	Pourcentage
satisfied (AO01)	7	19.44%
neutral (AO02)	6	16.67%
not satisfied (AO03)	4	11.11%
not a user (AO04)	17	47.22%
Sans réponse	2	5.56%

Degree of satisfaction of the faciltie's instruments

Confocal

Réponse	Décompte	Pourcentage
satisfied (AO01)	23	63.89%
neutral (AO02)	5	13.89%
not satisfied (AO03)	0	0.00%
not a user (AO04)	6	16.67%
Sans réponse	2	5.56%

And your satisfaction about:

Réponse	Décompte	Pourcentage
satisfied (AO01)	32	88.89%
neutral (AO02)	2	5.56%
not satisfied (AO03)	0	0.00%
Sans réponse	2	5.56%

Current availability
Of technical staff

Réponse	Décompte	Pourcentage
satisfied (AO01)	22	61.11%
neutral (AO02)	11	30.56%
not satisfied (AO03)	1	2.78%
Sans réponse	2	5.56%

Quality and
quantity of
informations

Do you need to use Machine learning in the next few months

Réponse	Décompte	Pourcentage
Oui (Y)	17	51.52%
Non (N)	16	48.48%
Sans réponse	0	0.00%

Your global appreciation about the Imaging facility

Réponse	Décompte	Pourcentage
very satisfied (AO01)	12	36.36%
satisfied (AO02)	19	57.58%
moderately satisfied (AO03)	2	6.06%
no satisfied (AO04)	0	0.00%
Sans réponse	0	0.00%

New investments

Réponse	Décompte	Pourcentage
Réponse	24	72.73%
Sans réponse	9	27.27%

Identifiant (ID)	Réponse
3	nno
7	No
8	Not this year
9	No
11	No
14	no
15	No, waiting for the lightsheet (hood of the lightsheet)
16	no
17	no
19	Yes, new Confocal
20	No.
21	No
22	An imaging facility fluorescent binocular for sorting or dissecting would be useful in particular for flies
27	no
28	A slide scanner would allow me to save huge amount of time
30	no
32	No, I don't have any specific needs for the moment.
33	binoculaire confocale SVP
36	no
38	yes, confocal LSM 880
41	no
42	no
43	no
44	I wish we could invest more in non-linear microscopy (two-photon with excitation of red fluorophores, second and third harmonic, etc), coupled with efficient incubation systems (temperature and Co2) for live imaging.)

To resume

- Good availability of technical staff
- Communication to improve
- Spinning disk problems : ?
- Deep learning needs to identify
- New microscopes : confocal, two photons, slide scanner...
- Good global appreciation of the facility

Electron microscopy

- New fares
- New equipment
 - Critical point drying
 - Binocular scope + caméra
- Works & access

New fares

- Table



Type microscope	Coût horaire (heure) HT				
Microscopes	T1	T1 bis	T2	T3	T4
FEI TECNAI	585,26 €	557,39 €	290,37 €	90,44 €	82,59 €
FEI TENE0	1 549,19 €	1 475,42 €	1 208,40 €	63,13 €	32,57 €
ULTRAMICROTOMES	330,57 €	314,83 €	47,81 €	42,59 €	25,82 €

- T4: Teams from PICsL laboratories (IBDM, CIML, INMED, IMM, Institut Fresnel)
- T3: Teams from laboratories outside the PICsL perimeter
- T2: Other public users
- T1: Private users

- Application

- For every project started after the 1st of january 2022

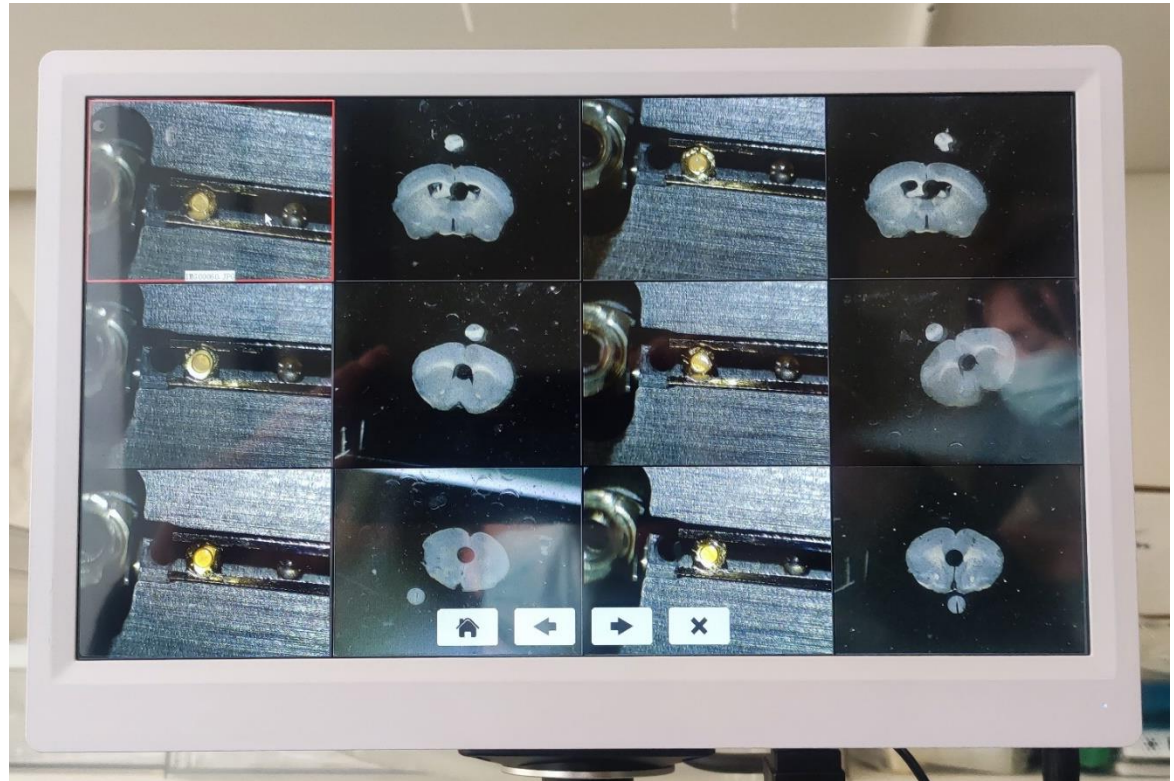
New equipment : Critical point drying (Leica)

- Used for SEM sample preparation
 - Replacement of a 40+ device (automatization, safety, reproducibility)
 - Conventional prep
 - Development of correlative SEM-TEM or SEM-SEM approaches
- Purchase
 - Purchase comitee (20k€)
 - Facility budget (10k€)



New equipment : Trinocular scope + caméra (Euromex)

- Small samples handling
 - Light from below and above
 - Equipped with a camera and dedicated screen (group work, quality approach...)
 - Doubling of equipment for safety reasons (+/- epoxy resin)
- Purchase
 - Facility budget (2k€)



Works and access

- Timeline



Aïcha : 06 95 10 47 95 – Fabrice : 06 64 31 32 01 – Nicolas : 06 42 91 53 19

Electron microscopy

- Any questions ?
- Any suggestions ?
 - New methods ?
 - New tools ?
 - ...

New project UAR :
Unité d'Appui à la Recherche

Multi-Engineering Platform

Objectives :



- **Gather engineers from complementary fields of expertise**
Complement the expertise of the existing platforms personnel.
Encourage multidisciplinary.
- **Help CENTURI teams with their research**
From hardware development to the acquisition, management & processing of data.
- **Promote interactions between CENTURI teams**
By the organization of events and training.
- **Facilitate the development of industrial partnerships**
By filling the gap between proof-of-principle and early prototypes.

CENTURI multi-engineering platform



Muhammad Asif

Bioinformatics & Data analyst



Sophie Brustlein
(Alumni)

Optics & Biophotonics



Benoit Dehapiot

Image processing & Data analyst



Guillaume Gay
(Alumni)

Database management & Data curation



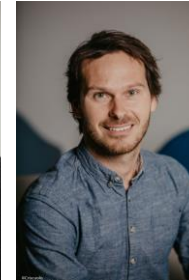
Mathias Lechelon

Mechatronics



Stefania Sarno

Data neuroscientist



Thomas Vannier

Bioinformatics & Data analyst



Thomas Boudier

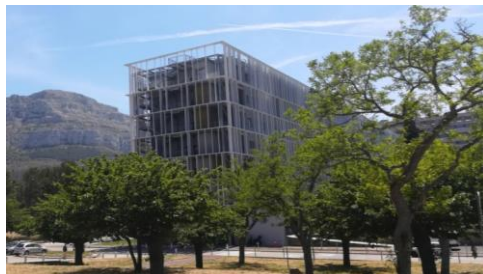
Image processing & Data analyst

- Optics & Biophotonics
- Database management

Call in progress

- Microfluidics Pierre Bohec (February)

33 6 17 22 28 83

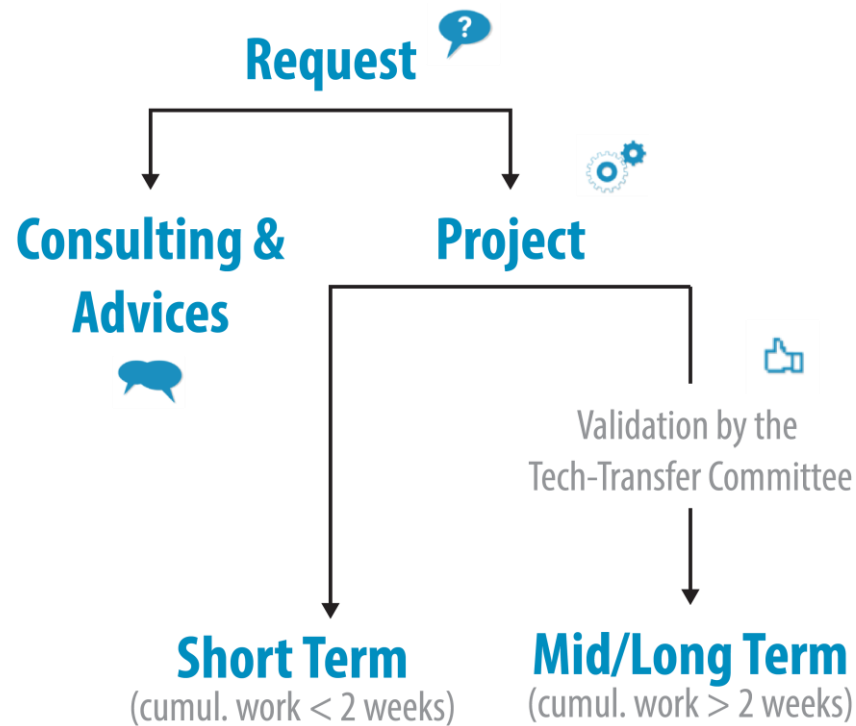


 <http://centuri-livingsystems.org/multi-engineering-platform/>

 engineering@centuri-livingsystems.org



Our process



Public project informations

online PDF

These infos will be published on our website

- Contact and current situation
- Project title
- Project summary (200 words)

Briefly describe the scientific background, the type of data and the required analysis, tools or services.

Confidential project informations

- Project description

Please provide a more complete description of your project, focusing on the technical aspects of the required analysis, tools or services.

✓ Terms & conditions agreements

- CENTURI engineers who collaborate to produce significant tools/analysis/services should be listed as co-author on any publication arising from the project.
- Please acknowledge CENTURI and the multi-engineering platform in your publications, talks and seminars to promote our visibility.

Training and community building

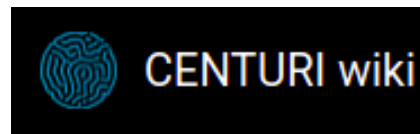
- Engineers are engaged in training program and events.



CENTURI Focus on imaging 2021

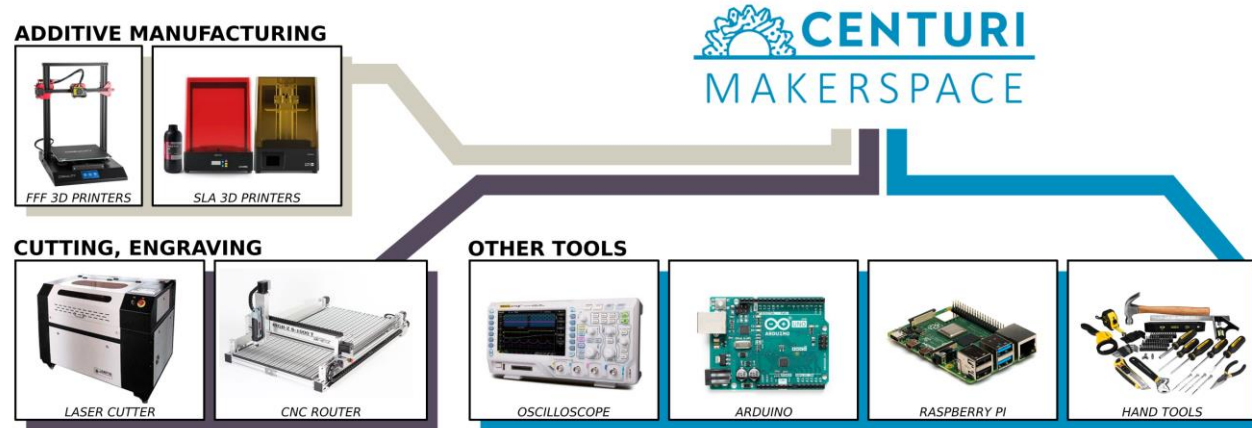


- Sharing methodologies and tools.



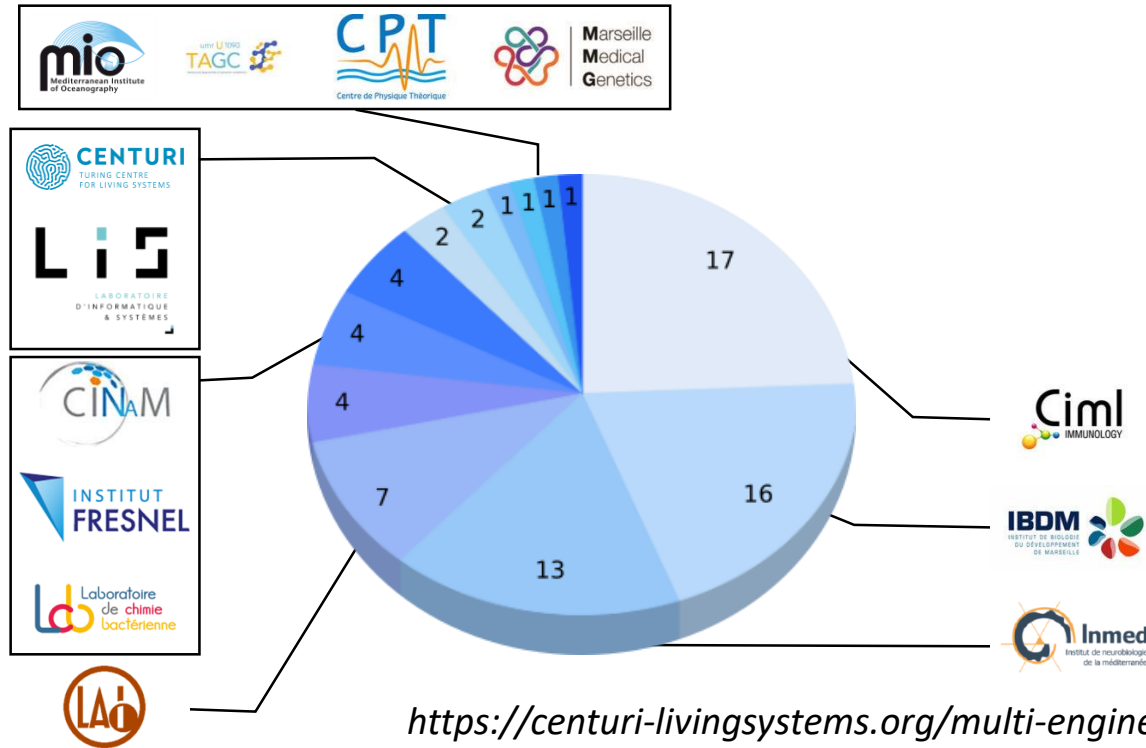
Setting up common infrastructures

- **Makerspace (AKA fab lab).**
CENTURI workshop open to CENTURI members.



- **Server in the Luminy data center**
To host research applications and development databases

Collaboration



<https://centuri-livingsystems.org/multi-engineering-platform/>

- Started in 2019
- Different locations
 - Luminy
 - La Timone
 - Joseph Aiguier
 - Saint Jérôme
- 73 submitted projects
 - 34 delivered
 - 39 ongoing
- 13 training actions
- 15 public interventions



Functions / Expertise in the UAR (Unité d'Appui à la Recherche)

- image processing and analysis
- bioinformatics
- deep learning
- mechanics
- microfluidics, microfabrication and microengineering
- electronics
- optical instrumentation
- data curation
- software development