AAPG2020	**WATEM**		**PRC**
Coordinator:	**Thierry EPICIER**	**36 months**	**448 k€**
CES42 "Capteurs - Instrumentation"			

WATER in a TEM

Table of staff of the project

Partner	Last Name	First name	Current position	Role and responsibilities in the project (max 4 lines)	Participation during the project (person/months)
IRCELYON, UCB Lyon	Epicier	Thierry	Research Director at CNRS (French National Center for Scientific Research)	-TEM / ETEM expert, coordinator -Responsible for the partner IRCELYON -Responsible of Work Package (WP) 6 -Participant to WP 1, 2, 4 and 5	18 (≈50% of full time)
	Nozière	Barbara	Research Director at CNRS	-Responsible of WP 4 -Participant to WP 5 and 6	7 (≈20% of full time)
	Cadete Santos Aires	Francisco José	'Chargé de Recherches' at CNRS	-ESEM and ETEM approaches -Responsible of WP 5 -Participant to WP 2, 4 and 6	10 (≈30% of full time)
	Ehret	Eric	Assistant-Professor at UCBLyon 1	-ESEM approach -Study of aerosols -Participant to WP 3, 4 and 6	5 (≈15% of full time)
	Simonet	France	Engineer at CNRS	-SEM/ESEM expert -Participant to WP 2, 4, 5 and 6	3 (≈8% of full time)
	Massin	Laurence	Assistant-Engineer at CNRS	-Characterization of catalysis -Participant to WP 2 and 5	7 (≈20% of full time)
	Chatre	Clément	PhD study *(not financed by ANR)*	-Study of aerosols (measurements of surface tension of particles) -Participant to WP 3 and 6	4 (≈10% of full time)
MATEIS, INSA de Lyon	Masenelli-Varlot	Karine	Professor at INSA-Lyon	-ESEM / TEM expert -Responsible for the partner MATEIS -Responsible of WP 3 -Participant to WP 1, 4 and 6	4 (≈10% of full time)
	Roiban	Lucian	Assistant-Professor at INSA	-TEM specialist -Responsible of WP 2 -Participant to WP 4, 5 and 6	5 (≈15% of full time)
	Ferreira	José	Engineer at INSA-Lyon	-Electronics, nanomechanics -Participant to WP 1, 3 and 6	2 (≈6% of full time)
	Goudin	Christophe	Assistant-Engineer at INSA-Lyon	-Electronics -Participant to WP 3 and 6	1 (≈3% of full time)
MAJULAB, NTU Sg	Duchamp	Martial	Assistant Professor at NTU	-TEM, ECELL/Liquid microscopy expert -Responsible for the partner MAJULAB -Responsible of WP 1 -Participant to WP 3, 5 and 6	10 (≈30% of full time)
	Miserez	Ali	Associate Professor at NTU	-Specialist of biological materials -Liquid Cell Electron Microscopy -Participant to WP 1, 5 and 6	7 (≈20% of full time)
	? *(to be recruited)*	*(to be recruited)*	Post-doc at NTU, MAJULAB-LISION	-Participant to WP 1 and 6	12 (full time)

Evolution of the proposition compared to the pre-proposition of the first step

The consortium WATEM is constituted by three partners: MATEIS from University of Lyon - INSA, IRCELYON from University of Lyon - UCBL and MAJULAB (NTU component, Singapore).

Following preliminary exchanges with ANR representatives, we would like to inform the ANR and the evaluating committee of the following evolution which was been operated since the deposit of the preproposition during the first round of the current AAPG: **The coordinator of the project, Thierry Epicier, CNRS Research Director at MATEIS, has moved to IRCELYON since April 2020.**

This change, wished by Thierry Epicier himself, has been validated by the CNRS and both MATEIS and IRCELYON labs, which are close partners in the local Microscopy Federation CLYM (www.clym.fr).

According to this change, the following updates of the WATEM project have been performed:

- Barbara Nozière, CNRS Research Director previously responsible of the IRCELYON partner, has been replaced by the project coordinator Thierry Epicier
- A new scientific responsible has been designated for MATEIS partner: Prof. Karine Masenelli-Varlot.

Please note that this evolution does not induce any modification of the aim, objectives and scientific methodology (e.g. Work Packages and tasks), neither changes of the repartition of the requested financial aid, as they may have been described or suggested in the pre-proposition.

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The total amount of aid expected from the ANR was evaluated to **459 113 €** in the pre-proposition. It has been re-evaluated to **447 802.95 €**, that is a **decrease by 2.5%** in agreement with the ± 15% variation authorized by the ANR. This decrease is mainly due to a more accurate evaluation of equipment costs. It is worth underlying some important points regarding this budget justified item by item in §. **II.a-b)**:

(i) it comprises an important part related to the use of high performances electron microscopes with expensive running costs according to the costly maintenance contracts of these instruments (subcontracting, i.e. mostly internal billing).

(ii) The equipment budget is justified by the hardware required by the objectives of the project and linked to its instrumental vocation.

(iii) According to the implication of several permanent people, among them French researchers and technicians/engineers from the CNRS, staff expenses requested from the ANR are limited to a one year 'post-doc' position in Singapore and two M2 internships for the French partners

(iv) Several visits from the French teams to Singapore, and vice-versa, justify the significant amount devoted to missions in this collaborative study.

I. Pre-proposal's context, positioning and objective(s)

a. WATEM objectives and research hypotheses

The first objective of the project is to develop a novel approach in Environmental Transmission Electron Microscopy (ETEM) to observe liquids and nanomaterials immersed in liquids or in contact with liquid droplets or water vapor. The second objective is to apply this new microscopic approach to the study of interaction between water and aerosols. The associated key research subject to be addressed is the investigation of cloud formation in the atmosphere, which has numerous economic and societal implications, through weather forecast, as well as key climate implications.

Very interestingly, since the submission of the pre-proposition of this project only a few months ago, several international papers have been published on the two topics addressed by WATEM: observing the interactions of water vapor with solid surfaces [**LEV20, YUA20, UNO20**] and studying the condensation of water on atmospheric aerosols leading to the formation of clouds [**LEE20**, **SEM20**]. This underlines the relevance and strategic dimension ('hot topic') of the research proposed in WATEM and the highly competitive context.

Liquid and vapor water in an ETEM

Although the constant development of efficient closed cells on specimen holders for Liquid Cell Electron Microscopy (LCEM) has led to many advances over the last two decades, such cells still suffer limitations as their sealing membranes hamper comfortable tomography approach, optimized chemical analysis and signal-to-noise ratio (SNR). More importantly, such cells do not easily allow the **water (liquid) condensation-evaporation experiments or cycles** as required for the investigation of cloud formation by TEM. Whereas 'windowless' approaches already exist in electron microscopy (see section I.b): 'state of the art'), they are not optimally designed for water (vapor or liquid) experiments, which motivates the present project: building a versatile and easy-to-use holder dedicated to **wa**ter experiments in **TEM**.

As the main application of this development, we will focus on *in situ* condensation-evaporation cycles in the TEM to study the hygroscopic growth of sub-micrometer and nanometer aerosols in a controlled humid atmosphere. This aims to provide unprecedented experimental information for the understanding of cloud droplet formation in the atmosphere. In addition to the application to aerosol hygroscopicity explored in this project, this tool will open new future perspectives and insights in biology (living matter) and chemistry (colloids, aerogels/latex, crystallization).

Water – aerosols interactions and cloud formation

Predicting the formation of clouds in the atmosphere has been one of the main challenges in meteorology and climate science for over 30 years. This scientific gap has considerable impacts on weather forecasting and key human activities such as agriculture, water management, transports, etc…

As clouds are also the main cooling factor in Earth's climate, this gap is also the source for the largest uncertainties in the climate budget (Intergovernmental Panel on Climate Change, www.ipcc.ch). The challenges in predicting cloud formation are both computational and fundamental, and this project is addressing the latter. The new technical approach to be developed in this project will be applied to investigations of the formation of liquid clouds (or "warm" clouds"), which proceeds exclusively by the condensation of water onto aerosol particles, most of which are micronic or sub-micronic [**LOH16**].
Although the theory describing these processes has been established for over 80 years (Köhler's equation, [**KOH36**]), applying it to atmospheric particles, i.e. linking the particle composition to Köhler's equation, remains a major challenge. This is because experimental information on the "hygroscopicity" (water uptake) of these particles, a key parameter of Köhler equation describing their ability to take up water and grow with relative humidity (RH), has been considerably limited by the performance of techniques. Essentially, only one technique has been extensively used to study aerosol hygroscopicity so far: HTDMA ("Hygroscopic Tandem Differential Mobility Analyzer" [**SWI08**, **SEM20**][1]), which is based on the observation of aerosol populations, therefore introduces biases between the individual particle properties and measured averaged hygroscopicity. Environmental Transmission Electron Microscopy with water vapor represents therefore a powerful alternative to this conventional technique, since it allows the investigation of the properties and hygroscopicity of individual sub-micron particles as discussed later in the next section 1.b).

b. Position of the project as it relates to the state of the art

Study of 'water in a TEM'

Observing liquids, especially water, in a TEM has undergone numerous advances over the last decades thanks to the development of liquid cells mounted on specimen holders for conventional high-vacuum TEM. In most applications, the sample to be studied is sandwiched between two thin non-crystalline (SiN_x) membranes sealing a thin slab of liquid at atmospheric pressure (see **Figure 1a-b)** and recent reviews [**ROS17, DEJ19, PU20**][2]). Despite irradiation effects mainly due to radiolytic reactions hardly avoidable (see **Figure 1c**), LCEM is of high interest for many subjects such as crystallization in liquids, biology applications (see **Figure 1d-f)** and references in previous reviews). Nevertheless, LCEM suffers from other intrinsic constraints inherent to the presence of sealing membranes, SiN_x current thin films or graphene sheets as encapsulating media (e.g. [**YUK12**]). These barrier-layers limit LC performances and make them clearly inappropriate for the type of experiments proposed in this project (condensation cycles). A very recent work shows that introducing water vapor in a closed cell is however possible, but it requires a complex setup to avoid unwanted condensation in the tiny (a few dozen of microns in diameter) capillaries where the liquid circulates [**UNO20**].
Investigating liquids and especially water without a closed cell (or windows) in an electron microscope is an issue because of the required high vacuum in its column: at a given temperature T; the environmental pressure P must be lower that the equilibrium pressure vapor so that the compound stays in liquid state. **Figure 2a)** is relevant of the case of water: at a pressure of typically 5 -10 Torr (1 Torr = 1.33 mbar), water or hydrated objects are thermodynamically stable when the sample is cooled down to typically 1 to 10°C. We will briefly survey the cases of SEM and TEM.

- **Windowless liquid Environmental SEM (ESEM)**

Liquid electron microscopy without a closed cell has been readily available in Environmental Scanning Electron Microscopes (ESEM) for almost three decades (see e.g. [**DAN91**, **HOF04**, **BOG05**, **STO08**]). If

[1] There is a very large literature on the use of HTDMA studies of aerosols; we just cite here two indicative reviews.
[2] Liquid Electron Microscopy is a very active domain with a very high number of publications coming out every month; as a specific illustration of this overall interest of the community, a Gordon Research Conference was recently organized entitled 'Liquid Phase Electron Microscopy', January 26-31, (2020), Lucca (Barga), IT (https://www.grc.org/liquid-phase-electron-microscopy-conference/2020/). In the interest of space, we only cite here the recent reviews **ROS17, DEJ19** and **PU20** as a short but very representative selection of these works and advances in the domain.

environmental conditions correspond to the {6-15 mbar, 0-11°C) P,T domain (**Figure 2a**), liquid water can be observed in the transmission mode under the form of 'microscopic' droplets of adjustable thicknesses and forming menisci over holes of classical TEM holey carbon grids (**Figure 2b**).

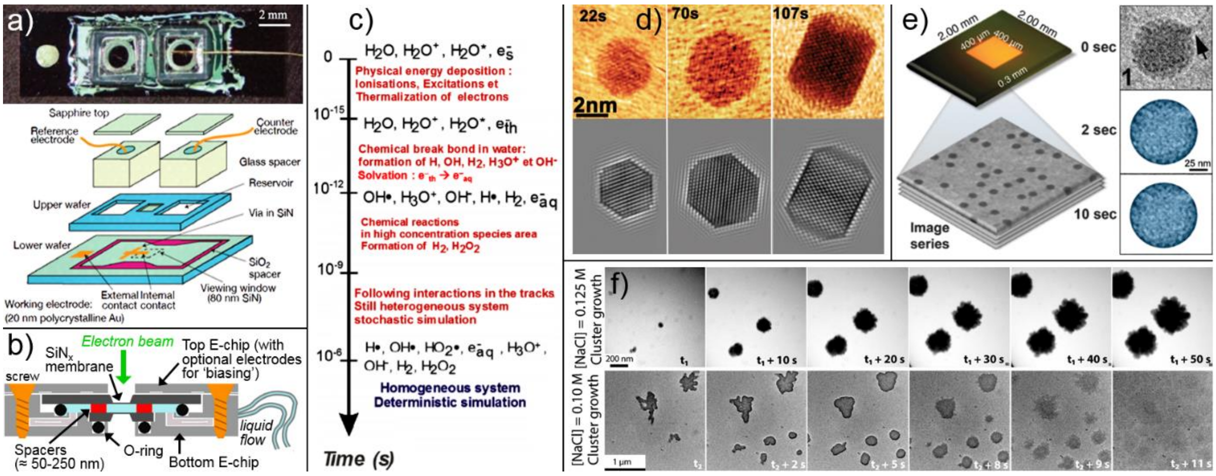


Figure 1: Illustrations of the state-of-the-art of LCEM. a) One of the first TEM liquid cell ion early 2000s' (from https://fmross.mit.edu/projects/liquid-cell-transmission). (b) Scheme of a closed Liquid Cell (adapted from commercial ads). c) Time scale of transient species produced by irradiation in pure water (adapted from Fig. 1 in [**BAL19**]). d) Growth of a Pt crystalline nanoparticle in a Pt-containing solution (adapted from Fig. 1 in [**LIA04**]. e) Imaging of purified rotavirus (RV) double layered particles (DLPs) with viral mRNAs transcripts (adapted from fig. 2 in [**VAR15**]). f) Time-lapse *in situ* sequences showing the formation and growth of protein nanoclusters in aqueous liquid. Top row: [NaCl] = 0.125 M. The nanoclusters did not dissolve over time and remained stable. Bottom row: [NaCl] = 0.10 M. Clusters first grew then disappear; the lower contrast of protein nanoclusters show that they are less dense when formed in a lower salt concentration (adapted from Fig. 3 in [**LEF19**]).

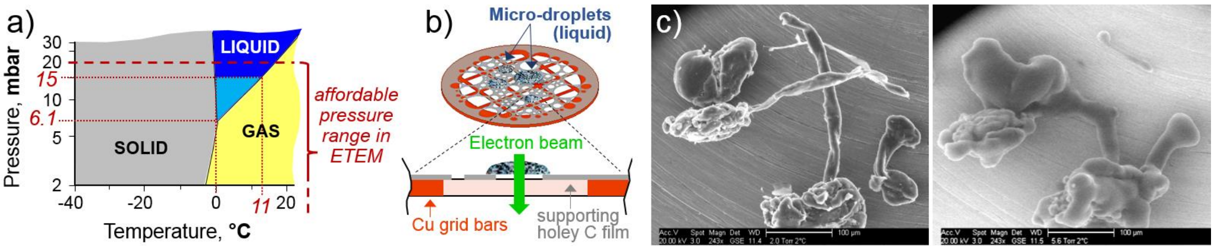


Figure 2: Liquid water in a TEM. a) Thermodynamic diagram showing the relative stability of water phases (liquid, solid, gas). A reasonable {6-15 mbar, 0-11°C} {P,T} domain for observation of liquid water in ETEM is shown. b) Principle of observation of micro-droplets of water hanging on a holey carbon film (TEM Cu grid) cooled down in the adequate (P,T) domain. c) Hydrated objects in the ESEM at CLYM, Lyon. Sequence of *in situ* hydration of an adjuvant (cellulose ether) user for water retention in buildings renovation renders (ESEM experiment at 2°C in a wet atmosphere from 2 to 5.6 Torr [**BER04**]).

In this geometry, the electron beam does not cross the sealing membranes of the Liquid Cell. At worst, it crosses only the supporting carbon film which contributes negligibly to the image contrast in comparison to the SiN_x membranes of a Liquid Cell because of its lower mass-thickness value. This provides much better imaging conditions than in LCEM (tilt, SNR, lateral extension, easy-to-use), although the ESEM spatial resolution is less than in a TEM. In addition, the liquid-vapor equilibrium is maintained dynamically through the thermodynamic stabilization of the droplets/menisci. This direct liquid-gas interface makes possible the study of the water condensation on aerosols at submicrometer / nanometer resolution, which is impossible in closed cells. 'WET-Microscopy' has been carried out successfully at the state-of-the-art since many years in an Environmental SEM (FEI/Thermo Fischer Scientific™ ESEM such as installed at MATEIS-CLYM [**BOG05, MAS14**]). One of the first reports on the hydration and dehydration of aerosols concerned $NaNO_3$ particles [**HOF04**]. Similar experiments showing the interaction of samples with condensed humidity were also performed in the ESEM at

CLYM, Lyon **(Figure 2c)**. Most studies consist in 2D observations since images from the microscope are projections of 3D objects. However, a 3D description of the internal structure of heterogeneous aerosols, as well as their morphological evolution during water uptake would be valuable information to obtain in situ. Interestingly, ±70° tilt tomography has been performed in WET-SEM[**XIA19**], and it demonstrated that Brownian motion or mobility in the liquid is not necessarily a crippling obstacle. Note that such an 'open cell' configuration in both SEM or TEM (see below) presents significant advantages for tomography compared to the current TEM close cells, where the tilt amplitude is typically limited to ±45°. Such a low tilt strongly degrades the quality of the 3D reconstructions [**DEA19**]. French partners of WATEM have a privileged access to a dedicated FEI-Titan ETEM instrument at CLYM Lyon (www.clym.fr) where a gas pressure of up to about 20 mbar can be maintained around the specimen in the pole pieces gap [**JIN12**]. Therefore, experiments similar to those conducted in ESEM can be performed in the ETEM with liquid water, including the condensation of water vapor, if the sample is cooled down to a few °C at a water pressure of a few mbar. Details are given hereafter.

- **Windowless liquid or 'WET' Environmental TEM (ETEM)**

This approach has been pioneered by P.L. Gai who developed a liquid injector in a sample holder fitted in a home-made modified ETEM version of a commercial TEM operating at 200 kV [**GAI02**]. In this setup, water could not be used as no cooling system was attached to the holder. Instead, polymerization experiments were performed with adiponitrile $C_6H_8N_2$ (liquid at room temperature even in the partial vacuum of an ETEM). More recently, *ionic* liquids, with a much higher vapor pressure than water, were also observed in a high vacuum TEM [**MIY17**].

Concerning water, interesting studies, in which water vapor was introduced in a first generation ETEM for the purpose of studying aerosol hygroscopicity, were reported about 15 years ago [**WIS05**, **FRE09**, **POS13**] (see below and discussion in the next sub-section). Cavalca and co-authors introduced water vapor in the ETEM to study light-induced photocatalytic reactions [**CAV12**]. More recently, Cassidy et al. [**CAS17**] have elegantly worked with a highly saturated atmosphere but without condensing water due to the absence of adequate cooling device. The catalytic reaction of methane steam reforming was also tested by our group [**EPI18**] in the ETEM using a CH_4-H_2O mixture up to 650°C. In a recent work [**YUA20**], a similar approach was used to inject in the ETEM a mixture of CO and H_2O vapor at a gas pressure of 5 mbar and a temperature of 700°C. While studying the water-gas shift catalytic reaction, they investigated TiO_2 (001) surfaces at atomic resolution and showed the evolution of twin protrusion hydroxyls complexes. However, no direct control of the chemical adsorption of H_2O molecules was performed. As stated above (**Figure 2a**), cooling the sample (holder) as performed in ESEM is required to condense water from the vapor or even study hydrated objects. This was indeed the method employed initially in pioneering experiments on various aerosols [**WIS05**]: the sample was mounted on a cryo-holder cooled down to the liquid nitrogen temperature and heated in a controlled way to a few degrees under a degraded vacuum (typically, 6°C and 7 Torr) to obtain a relative humidity (RH) of 100%. We performed similar feasibility tests in the ETEM in Lyon, see **Figure 3**. A beam-sensitive polymer-based nanocomposite (P(MA-co-BA) / imogolite $Al_2SiO_3(OH)_4$) was observed in liquid phase inside the ETEM using a commercial cooling liquid nitrogen (LN_2) holder **(Figure 3a-d)**. A very recent work using a similar dedicated ETEM [**LEV20**] has appeared since the submission of our pre-proposition in Oct. 2019, which attests of the high interest of this approach (see **Figure 3e-f**). In such experiments, the holder had to be heated from the LN_2 temperature to around a few °C, which corresponds to neither comfortable and easy-to-use, nor optimal conditions (lack of temperature stability and accuracy in the desired range - as quoted by Levin et al. [**LEV20**] -, induced vibrations that reduce the resolution). Nevertheless, all these results confirm the relevance of the propositions submitted in this project.

Microscopic investigation of aerosol properties and hygroscopicity

Aerosols are emitted into the Earth's atmosphere by a multitude of processes, including mechanical ones (sea spray, soil and desert dusts, volcanic ashes, …), biological ones (plant debris, pollens…) and human activities (combustion, car exhaust, tires, …). Some aerosols are also produced *in situ* in the atmosphere by the condensation or chemical reactions of gases. As a result, they encompass a wide range of particle size, from a few nm to tens of microns, and of chemical composition, including inorganic salts, minerals, organic materials, etc… (see for example Table 2 in [**PÓS13**]). Aerosols are present in all regions of the Earth, at concentrations between 10^2 or 10^3 particle/cm^3 of air for the cleanest ones (oceans, remote regions) to 10^6 particles/cm^3 air for the most polluted ones. But in the presence of moist air, only a few 100 particle/cm^3 will take up water and become cloud droplets, regardless of the initial aerosol concentrations. The current challenge in predicting cloud formation lies therefore in predicting which particles in these populations will take up water and in identifying the properties (mostly in term of particle composition) that drive this selection.

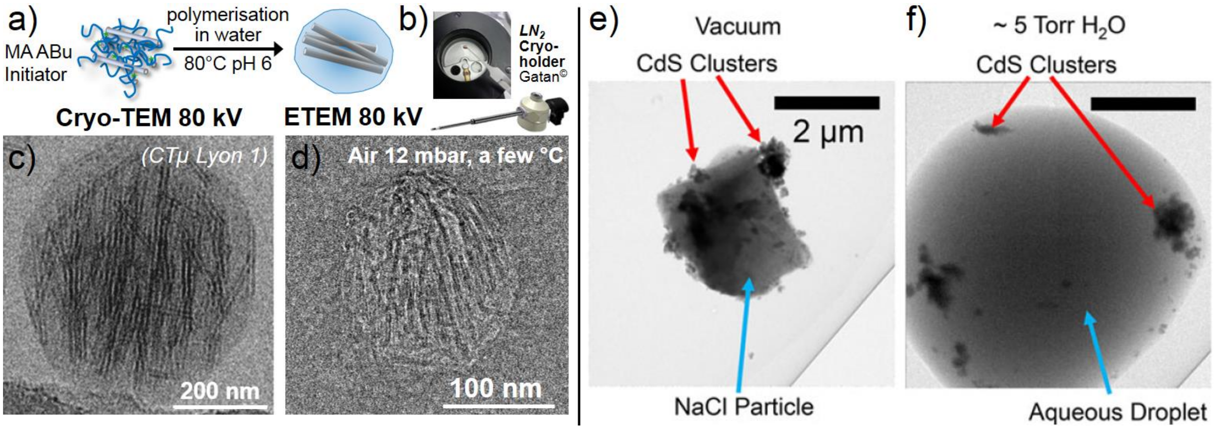


Figure 3: Wet and liquid microscopy in an ETEM. a-d) Preliminary 'WET-ETEM' experiment at CLYM-Lyon (holder kindly lent by CTμ platform Lyon). a) Brief description of the polymer-imogolite latex particles (see details in [**CEN14**]); b) liquid nitrogen (LN_2)-based cryo-holder; c): cryo-TEM image [**CEN14**] compared the image taken in WET-ETEM (d), T. Epicier, M. Aouine, F.C. Santos Aires, CLYM-Lyon, *unpublished*. e-f) Liquid microscopy in the ETEM at 300 kV (adapted from Fig. 3 in [**LEV20**]. CdS nanoparticles deposited on a NaCl particle are observed under high vacuum conditions. f) Water is introduced at a pressure of ≈ 4 mbar (5 Torr) as a usual gas in the ETEM, and condensation on the NaCl particle occurs at a cooling temperature of 2°C, allowing an aqueous droplet to embed the CdS nanoparticles.

As shown in a recent general review [**RIE19**], microscopy techniques such as AFM, SEM and TEM (including Energy Dispersive X-Ray spectroscopy EDX) have been widely used to analyze the composition, mixing state and morphology of atmospheric particles under dry conditions [**LAS15, MOF16, PAT16, HAM16, AUL17, XU17, KIR18, UNG18**]. In particular, AFM has been mostly used to investigate the interaction of aerosols with water, e.g. the surface tension of particles, another key parameter of Köhler equation, for laboratory- and seawater-generated aerosols [**MOR15, LEE17, EST17, LEE20**]. Environmental Electron Microscopy (EEM) has also been used to study the hygroscopicity of laboratory-generated [**HOF04**, **WIS05**, **FRE09**] and natural aerosols [**SEM07**, **FRE09**, **ADA11**, **POS13**], see **Figure 4**.

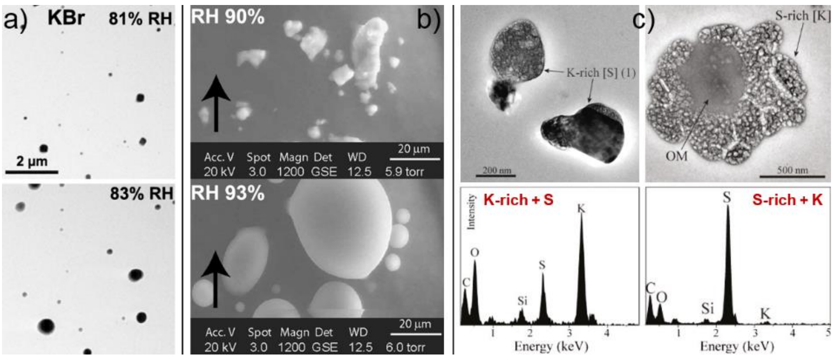


Figure 4: State-of-the-art of TEM and Environmental microscopy studies of aerosols. a) ETEM measurement of the DRH value for KBr aerosols (adapted from Fig. 2 in [**WIS05**]). b) Deliquescence ESEM study of KNO_3 while increasing the water vapor (adapted from Fig. 6 in [**FRE09**]). c) TEM morphology and chemical (EDX) analysis of particles collected in Northeast China during during winter haze days. OM means Organic Matter (adapted from Fig. 2 and 3 in [**XU17**]).

Several of these EEM studies [**SEM07**, **WI08, POS13]** reported some effects of the particle chemistry, composition and mixing state on their hygroscopic behavior, i.e. Deliquescence and Efflorescence Relative Humidities (DRH, ERH) values measured directly under wet atmospheres. As an illustrative example, DRH for KBr has been measured to 82% [**WIS05**], see **Figure 4a)**, whereas its value increases to 93% and > 90% in both ESEM and ETEM [**FRE09**] (see **Fig. 4b**) for KNO_3. Another important parameter is the mixing state of 'multiphase' aerosols as illustrated in **Fig. 4d**), which is known to modify the hygroscopic behavior of the particles (see for example in the case of KCl+KNO_3, KCl+K_2SO_4 compounds [**FRE09**]). These results remain however largely qualitative rather than quantitative. Furthermore, these early studies focused on minerals and inorganic salts, while organic material, much more critical for cloud droplet formation, turned out to be challenging to study with these techniques due to electron dose effects.

What should be done to improve the in situ characterization of aerosols under environmental wet conditions in ETEM?

While groundbreaking, the ETEM experiments performed over the last decades on aerosols exposed to humid atmospheres were limited on several aspects of key importance for cloud droplet formation:

- While they established a role of the particle composition and mixing state on its hygroscopic behavior [**SEM07**, **WI08, POS13**] the results were largely qualitative, thus precluding any parametrization that could be transferred into models. This was mostly because the mass ratio of the different phases inside these particles was not quantified and could not be quantitatively linked to their hygroscopic properties. By contrast, 3D tomography of the nanostructure of complex particles such as shown in **Figure 4c** would provide the exact volume of each phase which, coupled with EDX/EELS (Energy-Dispersive X-Ray spectroscopy and Electron Energy-Loss Spectroscopy) chemical analysis, would give their mass or molecular ratios. To the best of our knowledge, such tomographic observations has not been performed yet on individual aerosol particles. To achieve this, it will be necessary to increase the tilt capabilities of the specimen holder in the ETEM: neither cryo-holder cooled by a liquid nitrogen reservoir, nor closed liquid cells offer the convenient tilt range for adequate tilt series acquisitions. Therefore, we intend to develop for this purpose a new cooled tip compatible a commercial holder, which will allow an easy observation of the samples in contact with water vapor or directly immersed in liquid water (see section I.c) below).
- Efforts should be done to better study organic material, which is ubiquitous in atmospheric aerosols and plays important roles for cloud droplet formation. Optimizing both the energy of the incident electrons and the low-dose irradiation modes with the help of fast and sensitive modern cameras would thus give experimental access to this organic material with ETEM
- It would be worth working at high RH up to 100 %, contrary to hygroscopicity studies with conventional aerosol techniques (HTDMA) generally limited to RH ≤ 96 or 98 %. Such a limit is crucial in the study of cloud droplet formation because the most critical point in the particle hygroscopic behavior is activation, the maximum of their Köhler curve, which is close or slightly above RH = 100 % and represents the RH at which the particle grows spontaneously into a cloud droplet. Using the cooled micro-system that we intend to fabricate should help us in achieving such a goal, especially by fining tune conditions to allow very small increments of RH at the sample that have strong impact on particle growth at large RH (> 90 %).

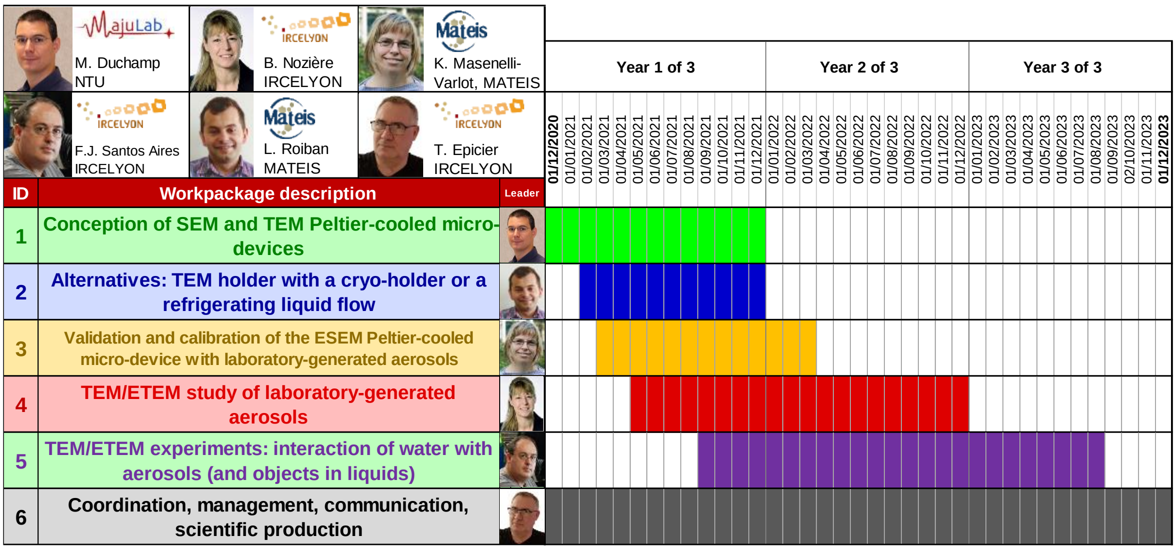
c. Methodology; pertinence to reach the objectives of the WATEM project

Plan of the work

The Gantt chart on next page illustrates the phasing of all steps and points out the scientific moderators of work packages organized around the following goals:

(i) Develop a disruptive Peltier-based hardware to study liquid processes inside an ETEM in an open cell environment, including a SEM version of the prototype (**WP 1**)

(ii) As alternatives to the Peltier cooler, investigate windowless liquid TEM approaches with other cooling means (**WP 2**)



- **(iii)** Validate the technical developments from WP 1 in ESEM by a calibration and study of the hygroscopic properties of laboratory-generated inorganic salts used as model aerosols; explore the tomography possibilities under WET conditions (**WP 3**)
- **(iv)** Perform a similar study in the ETEM as performed in the ESEM (WP 3). Include more complex laboratory-generated aerosols mixing organic and inorganic parts in order to validate 3D analysis of their internal structure (**WP 4**)
- **(v)** Proceed with studies of natural aerosols collected in Lyon, under both dry and wet conditions in the (E)TEM. Investigate their hydroscopic behavior near activation (large RH) and quantify their composition and mixing state with EDX/EELS and tomography. Explore perspectives to study nano-objects immersed in liquid with the Peltier-cooled micro device devised in WP 1 (**WP 5**).

Description of WATEM Work Packages (WPs) and Tasks

- **WP 1:** ***Conception of SEM and TEM Peltier-cooled micro-devices (responsible: Martial Duchamp, MAJULAB. Participants: Ali Miserez, post-doc to be recruited, MAJULAB; José Ferreira, Karine Masenelli-Varlot, MATEIS; Thierry Epicier, IRCELYON)***

Summary: - Task T1.1: fabricate a miniaturized Peltier-cooled device compatible with the ESEM
- Task T1.2: fabricate a miniaturized Peltier-cooled device compatible with the ETEM
- Task T1.3: ESEM prospective study of liquid-liquid phase separation of proteins

WP 1 is dedicated to the hardware development of a miniaturized Peltier-based tip which will be conceived and realized at MAJULAB (LISION lab.) who has the required expertise for such developments [**DUC14(b), DUC18, TAV18**] (e.g. white room operations, Focused Ion Beam - FIB - micro-machining, 3D-printing). The device will be attached to a commercial TEM holder available at CLYM and ETEM-compatible: the Wildfire© TEM holder from DENSsolutions™ (www.denssolutions.com). Such a TEM holder should comprise electrical feedthrough to regulate the temperature of the stage within the TEM and measure the temperature near to the observation area. We will take advantage of the latest development achieved in the field of TEM holder, i.e., the development of holders for MEMS-based chips. Such holders, first demonstrated for high temperature atomic resolution imaging, exhibit a very limited drift due to the low thermal mass of the observation area and allow a rapid variation of the temperature due to the low power that needs to be input. They have another under-explored advantage, which is to offer the possibility to easily and quickly exchange the chip by using needles for both the electrical connection and the fixation of the chip at the holder end. The project consists in realizing a dedicated miniaturized tip compatible with this holder but cooled by a Peltier micro-device, as illustrated by **Figure 5**. Micro-Peltier devices are available commercially from a few manufacturers, such as www.amstechnologies.com. They have compatible sizes, thermal (ΔT of about 70°C) and power supply characteristics (small current and voltage - power equal to a fraction of Watt - of the same order of magnitude of what is currently used for the Wildfire© holder at CLYM -).

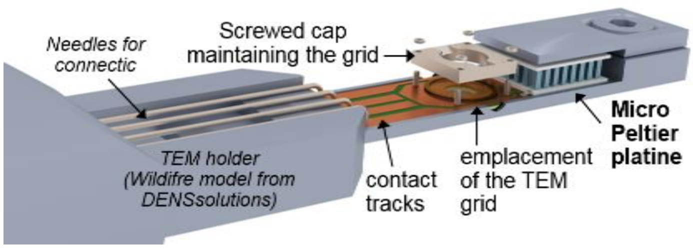


Figure 5: Design concept of a dedicated cooling micro-device (10 mm long by about 3.4 mm width) compatible with the Wildfire© TEM holder from DENSsolutions™ available at CLYM. The systems will accept usual TEM grids maintained in contact with the micro-Peltier on the right (cool side down, hot side maintained with a screwed fixing piece).

Apart from the ease to use this solution as compared to a liquid nitrogen cryo-holder, our cooling miniaturized tip should be well adapted for in situ tomography. Contrarily to most of the closed cells, and liquid nitrogen-based holders, our micro-device will be fabricated in order not to add any shadowing as compare to the DENSsolutions™ Wildfire© holder. The overall system, in which the miniaturized Peltier-cooled device will be fitted, will allow tilt up to 70°, the highest tilt value demonstrated in the course of the recent 3DCLEAN ANR project [**EPI19**]. The conception of such a miniaturized device (≈ 10 x 3 x 1.6 mm) for TEM application is extremely challenging. It has to overcome simultaneously three challenges: (i) the space constraint, (ii) keep the drift due to the external power injected in the Peltier stage that needs to be dissipated as low as possible, (iii) the lack of space in the pole piece and difficulties linked to the expensive access to the final instrumentation (ETEM) during the phase of development and testing.

To optimize our approach, a first task T1.1 will be to develop a larger version of the TEM micro-device for environmental SEM (ESEM). An ESEM is available at NTU, thus it will also be more convenient for development and testing. The ESEM version will have better thermal contact compare to the TEM version, thus providing a better heat sink. We will then decrease the size of the thermal contact down to the size used for the TEM geometry and check for its influence on the stability. Different geometry can thus be tested to minimize the overall drift. During this iterative approach, finite elements simulations will be run (subcontracting with a specialized company) to predict and optimize thermal gradients. The optimal geometry will then be used for the final version of the miniaturized TEM device (second task T1.2). During these developments, some preliminary observations will be made on aerosols at MATEIS (see WP 4) and on liquid-liquid phase separation of proteins at MAJULAB based on the recent expertise of both Pi and co-I at LISION (third task T1.3) as illustrated by **Fig. 1f)** [**LEF19**].

This Peltier-cooled miniaturized tip will constitute a stringent advance allowing further 3D approaches on moderately 'cooled' nano-objects even under dry conditions in Electron Microscopy. There are however a few risks that will be further discussed in section 1.d).

- **WP 2: Alternatives: TEM holder with a cryo-holder or a refrigerating liquid flow *(responsible: Lucian Roiban, MATEIS. Participants: Thierry Epicier, Laurence Massin, Francisco J. Cadete Santos Aires, IRCELYON)***

Summary: - Task T2.1: ETEM observation using a cryo-holder - Task T2.2: Explore cooling possibilities using a circulating refrigerating fluid

As we have seen from the literature [**WIS05**, **LEV20**] and our previous feasibility test (**Fig. 3a-d**), an alternative exists to observe water and water vapor using a 'cryo' LN_2 holder. Observations in this setup will constitute a first task T2.1. Another option not explored so far (to the best of our knowledge) consists in a simple circulation of a cooling fluid inside a specimen holder (second task T2.2). This second possibility has been rapidly tested using our commercial heating holder (from Gatan™) where a cooling circuit serves to safely cool down the tip when the sample is heated at very high temperature. Without any specific modifications, a target temperature of 10° has been easily reached during preliminary tests outside the microscope (in ambient air) by using a water+ethanol mix liquid cooled down to -3.5°. These alternatives solutions are less ideal in terms of vibrations and performances than the dedicated Peltier-based chip to be designed in WP 1. They will however be properly implemented in this WP 2 for comparison purposes and, to some extent, serve as a 'B-plan' in case of delay in the delivery of our prototype from WP 1.

- **WP 3:** ***Validation and calibration of the ESEM Peltier-cooled micro-device with laboratory-generated aerosols (responsible: Karine Masenelli-Varlot, MATEIS. Participants: José Ferreira, Christophe Goudin, MATEIS; France Simonet, Eric Ehret, Clément Chatre, IRCELYON; Martial Duchamp, MAJULAB)***

Summary: - Task T3.1: Peltier-based chip (WP 1) calibration in ESEM - Task T3.2: Overall and statistical characterization of large population of aerosols - Task T3.3: in situ 'WET-tomography' of aerosols

WP 3 is devoted to the determination of experimental conditions allowing a reliable characterization of water condensation/hydration on aerosols, using ESEM and laboratory-generated aerosols.
The first task T3.1 will consist in validating the design and fabrication of the ESEM prototype developed in WP1, then test and calibrate its cooling capabilities. We propose to carry out the validation and calibration on both ESEM instruments in Lyon and Singapore, for which running costs are far reduced compared to that of the ETEM. We emphasize that the MATEIS participants have a long experience with experiments involving liquid water in ESEM. The microscope at CLYM is already equipped with an electrical feedthrough flange from DEBEN for its connection to the sample holder controller. The comparison of the (P, T) values with values obtained using the standard Peltier stage will allow the determination of a calibration curve for the miniaturized cooled tip developed in WP 1.
In a second task T3.2, sub-micron aerosol particles made of material for which the hygroscopic behavior is well documented (pure NaCl or $(NH_4)_2SO_4$) will be studied. These aerosols will be generated by classical methods such as spray or atomization of aqueous solutions of the salts, the concentration controlling the size distribution obtained. Then, the DRH and ERH values obtained will be calibrated with methods and results from the previously cited literature (e.g. [**WIS05**, **FRE09**]). Attention will be paid to irradiation effects: for instance, the influence of the electron dose rate and image acquisition dwell time on the stability of both aerosols and water droplets will be quantified. The behavior of irradiated aerosols will be compared with that of non-irradiated ones; these behaviors will then be further compared to what is observed in the ETEM (see WP 4). ESEM should allow a characterization with very good statistics owing to the large field of view.
The third task T3.3 will focus on determining the possibilities to perform electron tomography on aerosols deposited on the cooled tip to evaluate their 3D morphology. We will use the existing home-made device available at CLYM and allowing for rotation of the sample over 360° [**MAS14**, **XIA19**], although the rotation amplitude will be limited by the supporting tip itself, see WP 1.

- **WP 4: TEM/ETEM study of laboratory-generated aerosols** ***(responsible: Barbara Nozière, IRCELYON. Participants: Eric Ehret, Thierry Epicier, Francisco J. Cadete Santos Aires, Laurence Massin, IRCELYON; Lucian Roiban, Karine Masenelli-Varlot, MATEIS)***

Summary: - Task T4.1: Peltier-based chip (WP 1) calibration in ETEM - Task T4.2: Hygroscopic properties of inorganic / laboratory-generated salts - Task T4.3: in situ nanoscale 'WET-tomography' of mixed aerosols

This WP is the (E)TEM equivalent to WP 3 dedicated to ESEM, consequently is has a very similar organization. The first task T4.1 aims at validating the design and fabrication of the Peltier-cooled micro device devised for the TEM, then test and calibrate its cooling capabilities in comparison with results obtained using alternatives in WP 2. The second task T4.2 will use the same model aerosol particles than in task T3.2 of WP 3 (pure NaCl or $(NH_4)_2SO_4$) to both perform a global TEM characterization and to measure DRH and ERH behavior under wet conditions in ETEM. Again, and more stringent for the TEM, attention will be paid to irradiation effects (see the risk analysis in section I.d) below).
Quantifying the role of the mixing state on hygroscopicity with EDX/EELS and electron tomography will be the ambition of the third task T4.3. For that, two sets of aerosols containing mixtures of inorganic salts (NaCl or $(NH_4)_2SO_4$) and organic compounds (for instance, oxalic or malonic acid) will be generated: homogeneously mixed aerosols, and aerosols with a salt core and organic coating, for which the volume ratio of the salt cores and organic coatings will be quantitatively determined with electron tomography. This ambitious approach will be extended to the case of natural aerosols, see WP 5.

- **WP 5: TEM/ETEM experiments: interaction of water with aerosols (and objects in liquids)** ***(responsible: Francisco J. Cadete Santos Aires, IRCELYON. Participants: Laurence Massin, Barbara Nozière, Thierry Epicier, IRCELYON ; Lucian Roiban, MATEIS; Martial Duchamp, Ali Miserez, MAJULAB)***

Summary: - Task T5.1: Collecting natural atmospheric aerosols
- Task T5.2: (E)TEM study of their 2D/3D structure, composition and hygroscopicity
- Task T5.3: Prospective exploration of WET-ETEM of nano-objects immersed in water

In this last experimental WP, all the knowledge and know-how obtained from the previous WPs will be applied to the exploration of more complex real objects, such as atmospheric aerosol particles (thus, by definition of unknown composition) and possibly other objects (see Task T5.3).

The first task T5.1 will be to collect natural aerosols. For this, micronic and sub-micronic atmospheric particles will be collected on TEM grids by placing these grids on the lower stages (0.056 - 1 μm) of a MOUDI impactor (as in [**MAR91**] for example[3]). For the scope of this project, the aerosols will be collected on the campus of Université Lyon 1, France (45° 47′ 00.3″ N 4° 52′ 02.9″ E), an urban environment, and at an altitude of about 180 m above sea level, similarly as in [**GER19**]. Recent works have shown that the micronic and sub-micronic aerosols at this site is diverse, including organic and non-organic material [**GER19**], thus suitable for the proof-of-concept proposed in this project.

The second task T5.2 will concern TEM (dry) and ETEM (wet) studies of the collected aerosols using the dedicated Peltier-cooled micro device devised in WP 1. The structure, composition and hygroscopicity of the collected particles will be investigated. A particular attention will be taken to the local chemistry of the aerosol particles that will be systematically analyzed by EDX and/or EELS both prior to water condensation and at different times while increasing the RH in situ in the ETEM.

Low irradiation doses and fast image acquisition (as permitted by the Oneview from Gatan© mounted on the ETEM) will be used to investigate the organic-based material (see the risk analysis in section I.d) below). RH will be increased in the ETEM up to or close to RH = 100 %, which will allow to capture the hygroscopic behavior of these particles near activation. We expect that accurate fast image acquisition during the gradual RH increase will allow for the first time to built the Köhler curves and determine the activation point (maximum of the curves) of individual sub-micron atmospheric particles, and to link them quantitatively with their internal composition and mixing state. This, in turn, will open the way to further development of rationale (equations) linking the composition and mixing state of the particles to their hygroscopicity, thus improving the model predictions of cloud droplet formation from aerosols.

In addition, as stated in WP 1, the newly designed Peltier-based device will be compatible with Electron Tomography, i.e. acquisition of tilt series up to about ± 70°. As for the laboratory-generated particles in task 4.3, electron tomography will thus allow to determine their internal structure and mixing state. More interestingly, fast tomography acquisitions in a few seconds, as developed in the 3DCLEAN ANR project [**BAN18**, **EPI19**, **KON19**], will be applied to, hopefully, perform rapid recording of images for 3D analysis during the water condensation process. Electron tomography in both ESEM and ETEM is here a challenge and 3D experiments will be an invaluable tool to better identify the onsets of condensation in different parts of a same particle, which is particularly relevant in the case of aerosols in a mixing state such as shown in **Figure 5d)**.

Lastly, we intend to explore possibilities of studying fully immersed nano-objects in liquid. As mentioned in section I.a) and illustrated in **Fig. 3a-d)**, the concept of the Peltier-cooled support allows the observation of fully hydrated samples, meaning also objects immersed in water. As a prospective third task T5.3 and depending upon the time available in the course of the project, we will perform preliminary observations on such systems (for example colloidal solutions), including the problematic of liquid phase separation in proteins already evoked in WP 1 (task T1.3).

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[3] Collecting aerosols, i.e. micronic or sub-micronic particles in suspension in the atmosphere currently requires the use of a dedicated device: a microorifice uniform deposit impactor (MOUDI) (see for example [**MAR91**]). An airflow is directed against flat impacting plates, which hold back the particles; a cascade strategy consists in using several stages (plates) allowing to discern the size of the collected particles down to the nanometer level.

- **WP 6: Coordination, management, communication, scientific production *(responsible: Thierry Epicier, IRCELYON. Participants: all)***

This WP concerns usual coordination tasks of PRC projects. The major point in the case of WATEM is the distance between French and Singaporean partners. The coordinator will pay a great attention to insure a continuous communication in between the few reciprocal visits of participants on one or the other side. Very regular e-meetings will then be organized to favor exchanges every 2 weeks at least.

d. Management of scientific risks

Experimental means to observe liquid and condense water vapor in the Environmental TEM

Realizing the Peltier-based chip depicted in **Figure 5** faces few issues that need to be evaluated.

(i) The developed tip should fit into the existing holder end, which is rather challenging according to the very limited available space at the tip of the holder. Whereas small Peltier stages with sufficient small dimension are available commercially (see **Fig. 5**), one of the challenge is to fit a conventional TEM grid having a 3 mm diameter. If it appears to be a too strong constraint, 2.3 mm TEM grids, also available commercially, will be used. Such grids are further easily observed in other conventional holders and other microscopes by sandwiching them between adequate 3 mm rings.

(ii) The spatial stability of the miniaturized Peltier-cooled device (along with a large tilt, see following point) is one of its main advantage. As compared to alternative solutions (see WP 2), the Peltier cooled stage will not suffer from vibration due to the presence of bubbling/moving liquid. The only source of drift we foresee is the consequence of thermal expansion due to additional power provided to the Peltier cooler. Firstly, this drift is relatively slow and in a given direction, thus quite easy to correct by image post-processing techniques. More importantly, the power needed to cooled down the Peltier stage is equal to a fraction of Watt and of the same order of magnitude of what is currently used for the Wildfire© holder at CLYM to heat the MEMS chip while still allowing atomic resolution imaging. Thirdly, finite elements simulations will be run (subcontracting with a specialized company) to predict and optimize thermal gradients to reduce even further the consequences of thermal expansion.

(iii) Another main advantage of the miniaturized Peltier-cooled device, compare to existing cooling / liquid-cell systems, is to allow large tilt for comfortable acquisition of tilt series for tomography. The tilt range is essentially limited by the screwed cap fixing the grid shown in **Fig. 5**. Our preliminary design seems to be compatible with ±70° tilt demonstrated with the Wildfire© TEM holder from DENSsolutions [**EPI19**]; we will try anyhow to go as close as possible to such large 70° tilt value.

The most stringent questions refer to points (i) and (ii). Although we are confident about the pertinence of the chosen strategy, delay in the delivery (expected at the beginning of the second year (see Gantt chart above) is possible. In such case, experiments would still be possible using the methodologies reported in WP 2 partly tested at MATEIS (see **Fig. 4a**) and by other groups [**WIS05**, **LEV20**].

Irradiation damage in the ETEM

A major issue when studying the aerosols interaction with water (vapor and liquid) concerns irradiation effects due to the energetic electron beam in the TEM. This problem is indeed two: (i) at first, it is well know that radiolysis occurs in liquid water, promoting PH changes and generating undesired reactive species which will obviously introduce a bias in the natural evolution of what is being studied (see e.g [**SCH14**]). (ii) Secondly, aerosols mostly consist of soft matter which may easily be damaged during exposure to the incident electrons. There is no magic solution to circumvent these limitations to which all researchers currently publishing in the field of LCEM, and/or (E)TEM study of aerosols are dramatically exposed as we will be. One can however note that the effects linked to point (i) above might be less important in the ETEM than in a closed Ecell encapsulating a thicker liquid slab, thanks to the dynamic nature of the liquid-water equilibrium.

We will nevertheless employ the classical method of low dose imaging in order to control, meaning: minimize, as much as possible these effects (as discussed, for example, in the recent review [**KUN19**]).

At the same time, acting on are the accelerating high voltage may complement the benefits of reducing the electron total dose received by the samples. Specific tests varying the electron incident energy (from 300 to 80 and 60 kV as permitted by the ETEM after a manufacturer alignment at 60 kV) will help to evaluate these effects. Note that we expect that the lowest voltages might be useful for the initial characterization of aerosols in high vacuum conditions (as generally applied for soft 'polymeric-like' matter [**EGE12**]). However, it is obvious that this 'lower voltage' strategy is not a good solution for imaging water. According to numerous LCEM papers (some being reviewed in [**KUN19**]), observations at 120, 200 and even 300 kV can successfully be conducted on such materials, and even biological matter if low dose conditions are optimized. As mentioned in section 1.c) (WP 5), we can acquire fast TEM images owing to an optimized CMOS camera installed on the ETEM, the Gatan Oneview™ camera capable of acquisition times of 5 ms in a 1kx1k format. Even at 300 kV, short exposure times with a fast camera allows avoiding vaporization of volatile compounds such as the beam sensitive ammonium sulfate as demonstrated in [**VEG18**] (fig. S3 in SI). Additionally, STEM scans (down to 512x512 frames in ≈ 1 second) will approach reasonable low dose conditions, typically 10 [**VAR15**] to ≈ 1000 e-/nm² [**MIR12**] as used for biological samples.

Risks of missing pertinent information during the ETEM observations

Microscopic observations in general, and that of individual particles in special, always require a particular attention to the relevance of the studied phenomena and the derived insights to large collections of particles. Despite the very time-consuming ETEM experiments, particular attention will be taken to this point and statistically meaningful studies will be performed.
As mentioned in WP 3, the onsets of condensation in different parts of aerosol particles will be one the main experimental challenges that we face. The use of fast-tomography associated to the use of fast acquisition Gatan Oneview™ camera will minimize this risk.
Some phenomena are probably out of the reach of ETEM capabilities (at the present date); they deal, for instance, with the "organization" of the water and the diluted aerosol within the droplets. It has indeed been shown, using SFG, that in sub-micron droplets interfacial water molecules are much more organized than the molecules within the "liquid bulk" of the droplet [**SMO17**]; this type of observation will not be possible (for the moment) within the ETEM.

II. Organization and realization of the project

a. Scientific coordinator and his consortium and team

The scientific coordinator

Thierry Epicier is a CNRS researcher (DR1) affiliated to section 15 at the Chemistry Institute (Materials, Nano-materials Chemistry and Processes). He has a long-standing expertise in Electron Microscopy and especially TEM with recent developments in Environmental TEM and Fast 'dynamic' Electron Tomography [**BAN18**, **EPI18**, **ROI18**, **EPI19**, **KON19**]. He has been coordinating several administrative, financial and scientific actions during the last 2 decades (e.g., coordination of two ANR collaborative projects: CONTRA-PRECI 2006-2009: https://anr.fr/Project-ANR-06-BLAN-0205 and 3DCLEAN 2016-2019: ANR-15-CE09-0009).

Composition of the consortium: complementary of the partners

The project requires experts in Electron Microscopy and especially ETEM and ESEM (MATEIS, IRCELYON and MAJULAB/LISION), experts in atmospheric chemistry (IRCELYON, ATARI group) and experts in 'nano-conception' and liquid TEM (MAJULAB/LISION).

- **IRCELYON laboratory, umr5256 CNRS at UCBL (Univ Lyon), F**

IRCELYON (Research Institute on Catalysis and Environment in Lyon, ircelyon.univ-lyon1.fr) is a research center dedicated to the overall understanding of catalyzed reactions applied to industrial and societal issues in the fields of Energy, Chemistry and Environment. The IRCELYON team is mostly constituted by permanent staff: Laurence Massin (Ass. Ing.), France Simonet (Ing.), Francisco J. Cadete Santos Aires (CNRS Res.), Barbara Nozière (CNRS Res. Dir. She has 20 years of experience in physical chemistry and atmospheric chemistry in the USA, Sweden, Germany and France, of which 10 years in the study of aerosols and cloud formation [**NOZ14**, **NOZ15**, **NOZ16**]). A PhD student (Clément Chatre) currently at mid-thesis and currently working on aerosols characterization will be associated (but not financed) for four months to the project.

- **MATEIS laboratory, umr5510 CNRS at INSA-Lyon, UCBL (Univ Lyon), F**

MATEIS (MATerials EngIneering and Science, mateis.insa-lyon.fr) is a pluridisciplinary lab. with research activities on the three classes of materials (metals, ceramics and polymers) and their composites, incorporating their characteristics by volume and surface and their interfaces at various scales. The MATEIS team is mainly composed by permanent people from the 'Microscopy' group of the lab ('SNMS') and comprises Christophe Goudin and José Ferreira (Tech.), Lucian Roiban (Asst. Prof.), Karine Masenelli-Varlot (Prof.) who will be the responsible of this partner. The team is expert in WET SEM and liquid in ESEM since 15 years [**BOG05**, **MAS14**, **XIA18**, **XIA19**] which offers a guarantee of success of WP 4.

- **MAJULAB-LISION, umi3654 CNRS at NTU Singapore**

MAJULAB is an International Joint Research Unit based in Singapore (http://majulab.cnrs.fr). It gathers different laboratories from Université Nice Sophia Antipolis, Sorbonne Université, National University of Singapore and Nanyang Technological University (NTU). Its experimental and theoretical research activities focus on Quantum Science and Technology as well as Chemistry of Materials and Interfaces. The latter activity is the link with the present project; it includes part of the activity of LISION laboratory (Laboratory for In Situ and Operando electron Nanoscopy) involved in WATEM. The permanent staff of MAJULAB comprises Ali Miserez (Assoc. Prof.) and Martial Duchamp (Asst. Prof.). The team leader M. Duchamp also leads the LISION lab. which he created in 2016. LISION is also affiliated to the School of Mat. Sci. & Eng. at NTU, from which Chris Boothroyd (Dr.) and Hortense Le Ferrand (Asst. Prof.) will also participate but are not financed by the ANR. He has a strong experience in realizing accessories for TEM involving nanomechanics or nanoelectronics [**DUC14**, **DUC14b**, **DUC18**, **TAV18**]. He recently conceived a new aperture mechanism able to contact electrically MEMS devices with 50 connections for beam shaping, i.e. real-time modulation of the electron wavefront inside a commercial TEM (M. Duchamp et al., *submitted*). The team has internal access to EMs, cleanroom, FIB and 3D printing facilities.

MATEIS and IRCELYON are long-standing collaborators especially as main members of CLYM in the field of electron microscopy. The link with MAJULAB in Singapore, justified by the expertise of the LISION lab within this UMI in operando/*in situ* TEM, is further reinforced by an existing collaboration between M. Duchamp and T. Epicier through a French-Singaporean 'Merlion' project on *in situ* ETEM between LISION-NTU and MATEIS-INSA (2019-2020, project 43646XJ, www.voilah.sg/wp-content/uploads/2018/04/_Merlion-Results-2018-1.xlsx). The **gender balance** of the program is reasonable although not ideal (permanent staff 5F/8M), one woman is partner leader.

Table of implication of the coordinator and of scientific representatives of the partners in other running projects (next page)

Note: shaded cells correspond to running programs without any temporal intersection with the proposed project (2021-2024). If the WATEM program is validated, the scientific representative of the 3 partners will then have global implications in research projects respectively equal to 3 (MATEIS), 6 (IRCELYON) and 9.8 (MAJULAB) person.months *per year* including WATEM and during its duration.

b. Means to reach the objectives

Partner 1: IRCELYON (UMR CNRS 5526, UCBL Lyon1)

- **Staff expenses**

The IRCELYON team mainly relies on permanent staff; a PhD student will be associated to the project for a 4 months period in 2021 but not financed. A 6 months internship for a Master student is requested for an amount of 3 600 €. Staff expenses amount to 432 286 €; they are not included in the ANR supporting budget and contribute only to the complete costs of the project.

Non-permanent staff (6 months, Master internship), **IRCELYON**	**3 600.00 €**

Name of participant to the project	Person. month	Name of project call, financing agency, amount granted	project title	Name of the project coordinator	Start and End dates
Thierry Epicier	2	Campus France (French government), Merlion, France-Singapore; 8.27 k€	I.N.S.T.A.N.T. (project n° 43646XJ)	Matthieu Bugnet (French side), Martial Duchamp (Sg.)	01/01/2018 – 31/12/2020
	6	E.U.R. SLEIGHT, University of St-Etienne/UDL; 8.8 k€	DIffusion Of NanopartIcleS On Surfaces (DIONISOS)	Thierry Epicier	01/06/2019 - 30/11/2020
Karine Masenelli-Varlot	6	ANR PRC 2018; 254.7 k€	Measurement Accuracy – CAse of mechanical properties of Oxide nanoparticles (MACAO)	Lucile Joly-Pottuz	01/01/2019 – 30/06/2022
	3.5	ANR JCJC; 279 k€	StrUcturiNg and SETting processes of mineral cements for bone repair (SUNSET)	Solène Tadier	01/03/2020 - 29/02/2024
Martial Duchamp	5	NTU-SUG; 158.6 k€	Cryogenic Nano-Visualization of Electronic Transport in Situ and Operando a TEM	Martial Duchamp	01/10/2016 - 30/12/2021
	5	MOE AcRF Tier-1; 85.2 k€	Electron Wave Phase Modulation Using MEMS Devices Inside a TEM	Martial Duchamp	01/11/2017 - 31/04/2020
	5	MOE AcRF Tier-1; 76.1 k€	Atomic scale mapping of the electrical potential of ultrathin materials operando in a TEM	Martial Duchamp	01/11/2019 - 31/10/2021
	4	ASTAR AME; 267.7 k€	Study of length scale effects and interfaces on mechanical properties	Raju V. Ramanujan	01/03/2019 - 28/02/2024
	1	Merlion	In Situ ANalysis of Solid Oxide Fuel Cell in operation	Matthieu Bugnet (French side), Martial Duchamp (Sg.)	01/01/2019 - 31/12/2020
	12	MOE AcRF Tier-2; 535.9 k€	Visualizing Perovskite Growth to Unlock Optoelectronic Secrets	Martial Duchamp	01/07/2020 - 30/06/2023
	5	MOE AcRF Tier-2; 317.2 k€	ELECTrically Reconfigurable Optoelectronic materials through ionic modulation	Nripan Mathews	01/07/2020 - 30/06/2023

- **Instruments and material costs**

WP 1 relies on the realization of a dedicated chip cooled by a micro-Peltier device; this operation is devoted to MAJULAB (see below). However, for an easier calibration during WP 3 and current TEM observations during WP 5, a source meter is desired to control the chip (typically the Keithley device SMU2461, fr.tek.com/keithley-source-measure-units/keithley-smu-2400-series-sourcemeter, 9 500 €). Another equipment is needed to collect natural aerosols as described in the WP 3 paragraph (section I.c). The required budget is 28 000 €. The total for both equipments is then 37 500 €. As for MATEIS in work packages 2 and 5, IRCELYON will have to book the ETEM, a microscope facility at CLYM (internal costs). A large number of sessions will be required to conduct the delicate environmental observations under wet conditions, i.e. ¾ of a total of 96 days (meaning an average number of 24 days/year). The running costs for this internal billing are 52 992 € at a daily rate of 736 €. The last item in this budget for experimental costs concerns consumables (TEM grids, additional chips, gases and external drives for easy storage of numeric data) evaluated to 12 k€ for the 3 years period of the project.

SourceMeter Keithley SMU + Moudi impactor 110 NR (quote from TSI company, April 2020)	37 500.00 €
running costs ETEM at CLYM	52 992.00 €
consumables (TEM grids and chips, gases…)	12 000.00 €
Instruments and materials costs (including consumables), **IRCELYON**	**102 492.00 €**

- **Building and ground costs** - none -
- **Outsourcing / subcontracting (and intellectual property)**

WP 1 requires a validation of the conception of the Peltier-based cooled device from the point of view of its thermal behavior. This study will then be conducted by a private society based in Villeurbanne on the campus, EC2 (https://www.ec2-modelisation.fr/), which has the required expertise in modelling heat transfer and thermal equilibrium by finite elements calculations. Based on a quote established in

Sept. 2019 and with a reasonable security coefficient to allow several converging iterations on the architecture of the micro-device, an outsourcing budget of 8 280 € is anticipated.

subcontracting 'EC2' (quote oct. 2019)	8 280.00 €
***Internal and 'between partners' subcontracting,* IRCELYON**	**8 280.00 €**

- **General and administrative costs & other operating expenses**

This item mostly concerns travel expenses. We evaluate for all partners the following *typical* cost for participating to an international conference during 5 days: 2800 € (registration fees 600 €, flights 1 200 €, lodging and other on site: 200 €/per day). We evaluate that IRCELYON members will participate to 5 congresses (including the 20th International Microscopy Congress in South Korea, Sept. 2022, http://www.imc20.kr/index.php) for an amount of 14 000 €. One Visit in Singapore every of the 3 years for one person, plus two additional ones, on the following basis: 2 working weeks on site, 150 €/day, flights 1300 €); the amount for these missions is then 14 000 €. Reciprocal visits between French and Singaporean participants will first be a complement to regular internet meetings organized to insure a continuous communication during the project advances. They will also promote an internal dissemination of the know-how of each partner through 'live' meetings or seminars, and, in complement to easy shipping of small hardware parts (Peltier coolers and controller), they may allow to exchange specimen holders compatible with the FEI / Thermo Fisher Scientific instruments. Joined experiments will also be conducted. In addition, a budget for an open access publication is included (4 k€).

Participation to international conferences (two events/year during 2 years, one event the 3rd year)	14 000.00 €
Visits to LISION-Majulab at Singapore (five '2 weeks visits' during the duration of the project)	14 000.00 €
Fees for one open access publication (typically *Nature Comm.* or *Scientific Reports*)	4 000.00 €
General and administrative costs & other operating expenses, IRCELYON	**32 000.00 €**

- **Budget summary for IRCELYON**

The four previous categories lead to a total budget of 590 367.76 € when including 11 709.76 € of overheads and 432 286 € of staff expenses (permanent + non-permanent staff not financed by the ANR). The requested aid for IRCELYON amounts to 158 081.76 € as summarized below.

Non-permanent staff, IRCELYON	3 600.00 €
Instruments and materials costs (including consumables), IRCELYON	102 492.00 €
Outsourcing + internal and 'between partners' subcontracting, IRCELYON	8 280.00 €
General and administrative costs & other operating expenses, IRCELYON	32 000.00 €
Overheards (8% of the sum from above), IRCELYON	11 709.76 €
Requested aid, IRCELYON	**158 081.76 €**

Partner 2: MATEIS (UMR CNRS 5510, INSA-Lyon)

- **Staff expenses**

MATEIS team relies mostly on permanent staff; these staff expenses (71 160 €) are not included in the ANR supporting budget and contribute only to the complete costs of the project. A 6 months internship for a Master student is requested for an amount of 3 600 €.

***Non-permanent staff (6 months, Master internship),* MATEIS**	**3 600.00 €**

- **Instruments and material costs**

WP 2 relies on developing alternative and accompanying solution for water vapor and liquid ETEM with membranes; it requests to acquire a cryo-TEM holder (typically, double-tilt l_{N2}-cooled accessory and its controller from the *Gatan/Ametek* company: about 66 000 €). In work packages 1, 2, 4 and 5, MATEIS will have to book microscope facilities at CLYM (internal costs). The session prices are publically available on the CLYM website http://www.clym.fr/fr/node/349: for the ESEM involved in WP 4, the corresponding price is 262 € per day. A few consumables are also required. For the FIB possibly involved in WP 1 and 4, the daily cost for a CLYM partner (autonomous user) is 438 €. The total of the estimated microscopy running costs is 7 430 €. The last item in this budget concerns consumables for microscopy and for the electronic workshop (3 500 €).

DT TEM cryo-holder (Gatan/Ametek, cost evaluation asked to the company in March 2020)	66 000.00 €
ESEM (20 days, 262 €/day) and FIB (5 days, 438 €/day) sessions at 'CLYM-INSA', internal billing	7 430.00 €
consumables for TEM and electronic workshop	3 500.00 €
Instruments and materials costs (including consumables), **MATEIS**	**76 930.00 €**

- **Building and ground costs** - none -
- **Outsourcing / subcontracting (and intellectual property)**

In WP 5, most of aerosols to be studied in the ETEM are essential of a soft, organic-like matter. Although the microscope Titan ETEM 80-300 kV can be operated at the relatively low high voltage of 80 kV, an alignment at 60 kV would allow to a more accurate control of the possible irradiation effects of the particles, especially when observed under dry conditions prior to any water wetting (estimated cost 29 688 €). Finally, MATEIS will also book the ETEM during the progression of WP 2 and 5. We assign 25% of the total number of ETEM day sessions (32 days/year during 3 years to MATEIS and evaluate 10 days/year for the ESEM and 5 days/year for the FIB during 2 and 1 year(s) respectively.

ETEM alignment at 60 kV (outsourcing based on FEI/Thermo Fisher Sc. Evaluation, 2020/03)	29 688.00 €
ETEM sessions at 'CLYM-CNRS', billing between partners (32*1/4=24 days, 736 €/day)	17 664.00 €
Outsourcing, **MATEIS**	**47 352.00 €**

- **General and administrative costs & other operating expenses**

This item is similar to what is requested for IRCELYON, although slightly less missions are planned (see details in Table below).

Participation to international conferences (one event/year for 1 person)	8 400.00 €
Visits to LISION-Majulab at Singapore (one '2 weeks visit'/year for 1 person)	8 400.00 €
General and administrative costs & other operating expenses, MATEIS	**16 800.00 €**

- **Budget summary for MATEIS**

The four previous categories lead to a total budget of 227 416.56 € when including 11 574.56 € of overheads and 71 160 € of staff expenses (only permanent staff not financed by the ANR). The requested aid for MATEIS amounts to 156 256.56 € as summarized below.

Non-permanent staff, MATEIS	3 600.00 €
Instruments and materials costs (including consumables), MATEIS	76 930.00 €
Outsourcing + internal and 'between partners' subcontracting, MATEIS	47 352.00 €
General and administrative costs & other operating expenses, MATEIS	16 800.00 €
Overheards (8% of the sum from above), MATEIS	11 574.56 €
Requested aid, MATEIS	**156 256.56 €**

Partner 3: MAJULAB (UMI 3654, NTU Singapore)

- **Staff expenses**

Permanent staff expenses represent a total of 110 006 €; they do only contribute to the complete cost of the project and are not in the budget requested to the ANR. A post-doctoral fellow is required (one year position) for the development of the cooling micro-device/holder. His/her expenses amount to 65 178.36 € decomposed into 53 937.36 € of salary and 11 241 € as a gratification for living abroad (French CNRS regulations. Note that the post-doc will be recruited among local candidates already living in Singapore since more than 3 months, which leads to the 'living gratification' mentioned above and equal to 15% of the maximum).

Non-permanent staff (one year post-doc), **MAJULAB**	**65 178.36 €**

- **Instruments and material costs**

WP 1 relies on the realization of a dedicated miniaturized system cooled by a micro-Peltier device, which then needs to be bought together with its controller. Adequate products are commercially available (e.g., at www.amstechnologies-webshop.com/). During this prototyping step, the Peltier devices (several units needed) can almost be considered as consumables; the budget for the required equipment here is evaluated to about 6000 €. Further minor equipment and miscellaneous consumables for elaboration of the cooled device (PCB fabrication) will also be required; the total amount is estimated to 14 k€; it also comprises a few liquid cell chips (4000 € for two packs of 10) to be used as complementary LCEM experiments during WP 5.

Misc. TEM consumables + Cooled chip fabrication (Petlier devices and power supply)	14 000.00 €
SEM/FIB and TEM sessions, internal billing (resp. 20 days, 400 €/day and 16 days, 800 €/day)	20 800.00 €
Instruments and materials costs (including consumables), **MAJULAB**	**34 800.00 €**

- **Building and ground costs** - none -
- **Outsourcing / subcontracting (and intellectual property)** - none -
- **General and administrative costs & other operating expenses**

MAJULAB requires a budget for conferences and visits to France similar to that asked by the French partners. An open access publication is also expected.

Participation to international conferences (two events/year during 2 years, one event the 3rd year)	8 400.00 €
Visits to IRCELYON at Villeurbanne (five '2 weeks visits' during the duration of the project)	11 200.00 €
Fees for one open access publication (typically Nature Comm. or Scientific Reports)	4 000.00 €
General and administrative costs & other operating expenses, MAJULAB	**23 600.00 €**

- **Budget summary for MAJULAB**

The four previous categories lead to a total budget of 243 470.63 € when including 9 886.27 € of overheads and 110 006 € of permanent staff expenses (not financed by the ANR). The requested aid for MAJULAB amounts to 133 464.63 € as summarized below.

Non permanent staff (1 year post-doc), MAJULAB	65 178.36 €
Instruments and materials costs (including consumables), MAJULAB	34 800.00 €
General and administrative costs & other operating expenses, MAJULAB	23 600.00 €
Overheards (8% of the sum from above), MAJULAB	9 886.27 €
Requested aid, MAJULAB	**133 464.63 €**

Requested means by item of expenditure and by partner

The table below summarizes the various items constituting the budget requested from the ANR, i.e. **447 802.95 €** (a 2.5% decrease as compared to the initial budget of the pre-proposition).

		MATEIS		**IRCELYON**		**MAJULAB**	
Staff expenses		*Permanent staff (for complete costs only)*	***71 160.00 €***	*Permanent + non permanent not financed staff (for complete costs only)*	***432 286.00 €***	*Permanent staff (for complete costs only)*	***110 006.00 €***
		Non-permanent staff	**3 600.00 €**	Non-permanent staff	**3 600.00 €**	Non-permanent staff (post-doc, 1 year)	**65 178.36 €**
Instruments and material costs (including the scientific consumables)		DT-cryo holder + controller	**66 000.00 €**	SourceMeter Keithley SMU + Moudi impactor 110 NR	**37 500.00 €**	Misc. TEM consumables + Cooled chip fabrication (Petlier devices and power supply)	**14 000.00 €**
		running costs ESEM + FIB at INSA (internal)	**7 430.00 €**	running costs ETEM at CLYM	**52 992.00 €**		
		consumables for TEM and electronic workshop	**3 500.00 €**	consumables (TEM chips, gases)	**12 000.00 €**	running costs microscopes NTU	**20 800.00 €**
Building and ground costs		NA		NA		NA	
Outsourcing / subcontracting and rights for intellectual property		subcontracting Thermo Fisher Scientific	**29 688.00 €**	subcontracting 'EC2'	**8 280.00 €**		
		ETEM microscope running costs (external)	**17 664.00 €**				
General and administrative costs & other operating expenses	Missions	congresses	**8 400.00 €**	congresses	**14 000.00 €**	congresses	**8 400.00 €**
		missions to Singapore	**8 400.00 €**	missions to Singapore	**14 000.00 €**	missions to France	**11 200.00 €**
	Administrative management & structure costs	overheads	**11 574.56 €**	open access publication + overheads	**15 709.76 €**	open access publication + overheads	**13 886.27 €**
Sub-total		**156 256.56 €**		**158 081.76 €**		**133 464.63 €**	
Requested aid		**447 802.95 €**					

The staff expenses represent 16.2% of the total aid, the 'instrument and material costs', 'outsourcing/subcontracting' and 'general costs & other operating expenses category' account for 47.8, 12.4 and 23.6% respectively.

III. Impact and benefits of the project

Innovative nature of the project, ambitiousness and originality of the objectives and the methodology

A remarkable innovation of WATEM is its ability to easily observe water-solid interactions at nanometer resolution in an ETEM. Its ambition essentially lies in the challenges to apply this approach to aerosols for a better understanding of cloud formation at the sub-micrometer scale. Temperatures from 20°C to 0°C will be achievable, which correspond to the conditions at which liquid-water clouds are formed in the atmosphere [**LOH16**]. Freeing the studied object from the constraint of encapsulating membranes will also allow new approaches in Liquid EM. Furthermore, the availability of a 'soft-cooling' holder (as compared to traditional liquid-nitrogen cooling systems) offers great advantages regarding mechanical stability (to be investigated), cost or comfort of use (the device could in principle be adapted to holders for different microscopes). Another advantage is the adequacy of our Peltier-cooled micro-device to in situ Electron Tomography in a liquid or wet atmosphere, a critical challenge for nanomaterials in many domains. Accordingly, the partners of the WATEM consortium will share the rights of their work; they are in contact with the DENSsolutions™ company open to any more official future collaboration. Although exciting, our device requires a supporting holder and, more critically, an Environmental microscope to access the required controlled low vacuum insuring the thermodynamical stability of cooled water as a liquid. This very reduced market does not favor any sustainable development but initiatives in any commercial direction will remain envisioned at the end of the project.

Perspectives beyond aerosols

Another interest of WATEM is that all partners have matter for new studies in the field of latex or aerogels (MATEIS, e.g. [**XIA19**]), catalytic reactions involving wet atmospheres (IRCELYON, e.g. [**EPI18**]) or studies of liquid-liquid interactions (MAJULAB/LISION, e.g. [**LEF19**]). If supported, WATEM can then offer new perspectives of collaborative works. Also, new directions exist regarding aerosols, such as the impact of their hygroscopic properties on their optical properties and the interaction with light in the atmosphere (not studied here, see - for example – [**GUP15**]. Beyond the topic related to aerosols, WATEM is expected to open new future perspectives and insights in biology (living matter) and chemistry (colloids, aerogels/latex, crystallization) as quoted in the introductive section 1.a) of this document.

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Note: papers associating WATEM members are highlighted. 'Open access' papers are marked with ⓐ.

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