



HAL
open science

In operando study of gypsum crystal growth through in-cell environmental SEM

Alexandre Fantou, Annie Malchère, Anna Wozniak, Lucian Roiban, Sylvain Meille,
Karine Masenelli-Varlot, Solène Tadier

► **To cite this version:**

Alexandre Fantou, Annie Malchère, Anna Wozniak, Lucian Roiban, Sylvain Meille, et al.. In operando study of gypsum crystal growth through in-cell environmental SEM. *Journal of Crystal Growth*, 2024, 628, pp.127515. <10.1016/j.jcrysgro.2023.127515>. <hal-04883507>

HAL Id: hal-04883507

<https://hal.science/hal-04883507v1>

Submitted on 16 Jan 2025

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

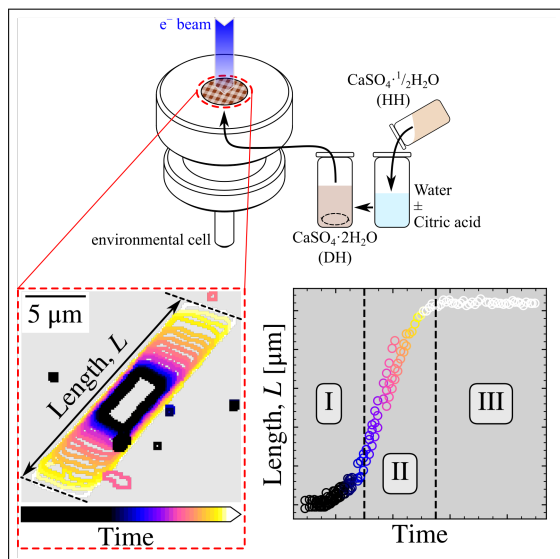


Distributed under a Creative Commons CC BY 4.0 - Attribution - International License

Graphical Abstract

***In operando* study of gypsum crystal growth through in-cell environmental SEM**

Alexandre Fantou, Annie Malchère, Anna Wozniak, Lucian Roiban, Sylvain Meille, Karine Masenelli-Varlot, Solène Tadier



Reproduced from doi: [10.6084/m9.figshare.23723673](https://doi.org/10.6084/m9.figshare.23723673) © (2023) A. Fantou, CC BY 4.0 license <https://creativecommons.org/licenses/by/4.0/>.

Highlights

***In operando* study of gypsum crystal growth through in-cell environmental SEM**

Alexandre Fantou, Annie Malchère, Anna Wozniak, Lucian Roiban, Sylvain Meille, Karine Masenelli-Varlot, Solène Tadier

- Evolution of gypsum plaster was analyzed during setting in conditions of use in SEM
- Kinetics of the growth of >100 gypsum crystals was computed from image analysis
- Growth rates of the gypsum facets were computed from crystallographic indexation
- A same gypsum crystal may experience different growth regimes along time
- A slower growth is experienced by crystals with thermodynamically unstable facets

In operando study of gypsum crystal growth through in-cell environmental SEM

Alexandre Fantou^a, Annie Malchère^a, Anna Wozniak^a, Lucian Roiban^a, Sylvain Meille^a, Karine Masenelli-Varlot^a, Solène Tadier^{a,*}

^aUniv Lyon, INSA Lyon, Université Claude Bernard Lyon 1, CNRS, MATEIS, UMR5510, Villeurbanne, 69621, France

Abstract

Thanks to their setting ability, hydraulic binder materials (*e.g.*, cements, plasters) are used for a wide variety of applications (*e.g.*, construction, medical). The setting reaction occurs through a dissolution – precipitation mechanism during which initial particles dissolve releasing ionic species from which new crystals precipitate. The development of such a microstructure governs the final properties of the material and are thus of uttermost interest.

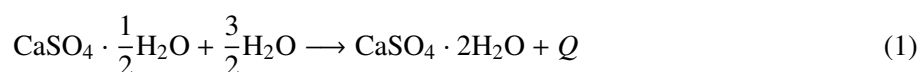
In this paper, the *in operando* study of the precipitation of gypsum crystals from the dissolution of bassanite particles using a specific cell for SEM observation is presented. Image analysis based on segmentation enabled to give quantitative results on the growth of crystals at global and local scales. Single crystal facets are indexed based on crystallography and literature results, which enables the quantification of crystal growth rates for the indexed crystallographic planes.

Keywords: Setting reaction, β - calcium sulfate, Microstructure, Image analysis, Crystallography, *in situ*

1. Introduction

Hydraulic binders (cements, gypsum plaster) are processed through dissolution-precipitation. While calcium sulfates are widely used in the form of gypsum boards for interior partitions, they are also often used as model materials for crystallization studies. This is explained by a relatively simple chemical reaction involving only crystalline phases, from common and cheap materials, with relatively large crystal sizes (microns to tens/hundreds of microns, possibly several meters in exceptional cases).

The processing of calcium sulfates starts with the dissolution of calcium sulfate hemihydrate (HH), also known as bassanite or plaster, followed by the precipitation of calcium sulfate dihydrate crystals (DH), also known as gypsum. The reaction usually takes place within minutes, with a complete hydration being reached in a maximum of one hour if no retardants are used. The hydration reaction is given here in eq. (1):



with Q the heat of reaction arising from the dissolution of calcium sulfate hemihydrate.

Historically, optical microscopy (OM) was first used to follow the growth of gypsum crystals, from supersaturated solutions in highly dilute systems [1, 2]. In the absence of additives, observed DH crystals were generally needle- and plate-like with wide size distributions. This method, although limited in terms of spatial resolution, enabled the quantification of various aspects of the precipitation of dihydrate crystals

*Corresponding author: solene.tadier@insa-lyon.fr

(*e.g.*, evolutions of shape and dimensions of crystals, effects of additives on crystallization, linear growth rates, kinetic order of the reaction rate). Combined with chemical analysis of the solution, it also provided information for measuring the induction period of the precipitation of DH crystals, during which nuclei are formed and start to grow by a "slow" process to form small crystallites [1].

To reach higher spatial resolutions, the evolution of the microstructure of calcium sulfate samples was studied with Scanning Electron Microscopy (SEM). To observe samples representative of industrial applications, *i.e.*, with typical liquid-to-solid mass (L/S) ratios below 1, the setting reaction was stopped by soaking and grinding samples into ethanol [3]; samples could then be studied once dried. The growth of gypsum crystals around HH particles followed by their entanglement at later stages were both evidenced, but no image analysis was performed for the quantification of the reaction extent. The need to stop the setting reaction (destructive characterization) impeded to follow the *in operando* evolution of one specific sample. Moreover, the preparation protocol may have affected the composition and morphology of the studied samples, as quenching crystallizing plaster in ethanol may stabilize HH with respect to DH, or even promote the formation of HH from already grown DH crystals, rather than preserving them [4, 5]. To solve this issue, the setting of gypsum paste was also monitored using a cell specifically designed for SEM observation, closed by a polymer membrane that is transparent to the electron beam, but water and air tight, protecting the setting paste from the chamber conditions (*e.g.*, vacuum) [6]. Thus, samples could be studied while they set, and with L/S ratios typical of industrial applications. In [6], the growth of needle-like gypsum crystals followed by their entanglement was regularly monitored within the first hour of reaction (after a dead-time of 17 min), while the growth of crystals was not completed yet. The study lacked from quantitative data describing the evolution of gypsum growth during setting reaction. Moreover, the influence of both the electron beam and the membrane on the development of the microstructure were still pending.

To study the early stages of gypsum nucleation, Transmission Electron Microscopy (TEM) was used either in High Resolution mode (HR TEM) after quenching of undersaturated solution [7], in cryogenic mode, or using a liquid cell to enable real time follow-up of the reaction [8]. In this latter work carried out in solution rather than in concentrated pastes, DH crystals were shown to nucleate on dissolving HH rod-shaped particles [8]. Still at the nanoscale, the motions of crystalline steps at the surface of DH crystals were investigated using Atomic Force Microscopy (AFM) [9–15]. However, this technique can neither be used for the characterization of setting pastes with representative L/S ratios, nor for the simultaneous monitoring of the dissolution of HH particles and the precipitation of DH crystals at a more global scale.

In situ monitoring of the setting reaction from dissolution of HH was also followed up at the mesoscale using X-ray tomography (μ -CT) [16, 17] and by combining 3D X-ray diffraction (s3DXRD) with phase contrast tomography (PCT) [18]. Advantages of these techniques are their non-destructive nature, the ease of sample preparation with no requirements in terms of paste fluidity or L/S ratio and 3D data providing information on the bulk of a sample. In all cases, the dissolution of HH grains, the formation of a porous network and the non-epitaxial growth of needle and plate-shaped interlocked DH crystals led to complex microstructures. With μ -CT (typical voxel sizes of 2.5 μm and of 0.65 μm to 0.163 μm , using laboratory and synchrotron X-ray tomographies, respectively), a "microstructural degree of reaction" was computed from the mean evolutions of volume fractions of dissolving HH particles and of precipitating DH crystals. At the single-grain scale, size measurements of individual HH and of DH crystals enabled to determine average dissolution and precipitation rates during setting reaction [16, 17]. The combination of s3DXRD with PCT enabled to identify reacting planes of the HH crystals and to monitor their evolution during dissolution. It was thus shown that the (100) and (010) planes of HH grains were preferentially dissolved [18]. However, nucleation of DH crystals could not be observed at this scale due to resolution limit, and observing their growth was particularly difficult during the early stages of the reaction [16–18].

In this paper, the same type of environmental cell as in [6] is used to monitor the precipitation of DH crystals from the dissolution of calcium sulfate HH particles *in operando*, until the microstructure seems not to evolve anymore. Then, image analysis of micrographs collected every minute enables to quantify the growth of DH crystals both at a global and at a local scale. The identification of growth directions and faces of DH crystals is also performed, enabling the measurement of the growth rates for each type of crystal planes along the reaction and in conditions of use (*e.g.*, L/S ratio, ...)

2. Materials and Methods

2.1. Gypsum plaster

Gypsum plaster ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) was obtained by mixing a HH powder ($\beta - \text{CaSO}_4 \cdot \frac{1}{2}\text{H}_2\text{O}$) with an aqueous solution. The liquid to solid mass ratio (L/S) was set to 0.6. The time at which the powder and the solution got in contact was defined as t_0 and corresponds to the origin of times for all experiments ($t_0 = 0$).

The calcium sulfate reactive powder was an industrial product from Mátra factory (Rigips, Halmajugra, Hungary). It was mostly composed of calcium sulfate hemihydrate (bassanite, $\beta - \text{CaSO}_4 \cdot \frac{1}{2}\text{H}_2\text{O}$) around 90 wt% and in lower extents of anhydrous calcium sulfate (anhydrite, CaSO_4), calcium carbonate (calcite, CaCO_3), silicon dioxide (quartz, SiO_2) and aluminosilicates.

Citric acid monohydrate $\text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$ (EMSURE Merck KGaA, Darmstadt, Germany, Catalog # 1002441000, Batch # K50959644) was used as a retardant of the hydration reaction. Two solutions were used, one consisting of pure deionized water (resistivity of $18 \text{ M}\Omega \cdot \text{cm}$) and the other one consisting of a 5.2 mM citric acid solution prepared with deionized water.

2.2. In situ SEM

Encapsulating cells QX202C from El-Mul (QuantomiX Ltd, Rehovot, Israel) were used to study the setting reaction of calcium sulfate hemihydrate with SEM under standard conditions (*i.e.*, atmospheric pressure, humidity). A scheme of the QX202C cell is illustrated in fig. 1(a). This cell, of inner volume comprised between $6.5 \mu\text{L}$ and $7.5 \mu\text{L}$, is closed by a top membrane, which is electron transparent but air- and water- tight. Thus, the wet setting paste, introduced inside the cell before its closure, is isolated from the vacuum of the SEM chamber.

Plaster paste was prepared by sprinkling 5 g of the HH powder in 3 g of the solution during 30 s. The obtained mixture was then manually mixed during another 30 s using a spatula describing movements in a figure of eight. Following the instructions of the QX202C cell furnisher, two volumes of $3.5 \mu\text{L}$ of the plaster paste were collected and deposited in the open encapsulating cell by means of a micropipette Microman M25 (Gilson, Middleton, Wisconsin, USA). Then, the cell was closed, and placed in the SEM chamber to start the observation as soon as possible, once the vacuum was ready.

The observation area was chosen by scanning the top of the cell (*i.e.*, grid, see fig. 1(b)) to check the homogeneity of the sample. Note the presence of the metallic grid, helpful to spatially locate different interesting spots.

The scanning electron microscope used is a ZEISS Supra 55VP SEM (Carl Zeiss Microscopy GmbH, Oberkochen, Germany) equipped with a Field Emission Gun. The acceleration voltage was set to 10 kV and the cell in which was introduced the setting paste was placed at a working distance of 10 mm. Images were acquired regularly using either the secondary electron Everhardt-Thornley detector, or a backscattered electron detector. The time step between two consecutive acquisitions was 30 s until a certain point where it reached 1 min when the microstructure seemed not evolve anymore. The time to record a micrography, denoted Δt_{acq} , lied between 3.8 s and 13.6 s (*cf.* table 1). Between two image acquisitions, the beam was

103 blanked to prevent irradiation damage in the sample. Two magnifications were used in order to image
 104 both the grid at lower magnification (pixel dimension of 744.3 nm) and the crystals at higher magnification
 105 (pixel dimension of 139.6 nm). The times of the first and last image acquisitions at higher magnification
 106 were denoted t_1 and t_{last} , respectively. t_1 corresponds to the dead-time required to get ready after mixing
 107 the HH powder with the liquid solution; it was typically of around 10 min to 15 min (*cf.* table 1). t_{last} was
 108 reached when no more significant evolution could be observed. The cell was then retrieved from the SEM
 109 chamber and stored at room temperature to make sure the setting reaction could proceed up to completion.

110 Some additional SEM observations were performed many days after (at t_{∞} , see table 1) at the very same
 111 locations with the same acquisition parameters (*i.e.*, working distance, acceleration voltage, magnification)
 112 to get information on the microstructure, once the plaster was completely set.

113 2.3. Image analysis

114 ImageJ / Fiji software was used to perform image processing and analysis [19]. All images of a same
 115 experiment (from t_1 to t_{last}) were first gathered into a stack (generating a timelapse, such as in [20]), in
 116 order to easily apply filters, and to account for the 2D microstructure evolution. First treatments on the
 117 stacks consisted of enhancing the contrast, denoising, and correcting background. Then the stacks were
 118 aligned to correct the sample drift. After a final median filtering, images were cropped in order to obtain a
 119 suitable size for observation and quantification. Typically, for the experiment illustrated in fig. 3, the final
 120 area was $(130 \times 70) \mu\text{m}^2$ (*cf.* table 1). This area was large enough to image a sufficient number of DH
 121 crystals (at least 100 of them) and thus to give a good representation of the evolution of the setting paste
 122 microstructure.

Table 1: Summary of all the experimental conditions for *in situ* SEM acquisitions. From left to right: citric acid concentration ([CAc.] in mM), experiment number, times of the first (t_1) and last (t_{last}) images, time of the infinite image (t_{∞}), acquisition duration of an image (Δt_{acq}) and surface area of the region of observation. Times of the first, last and of the infinite images are given with respect to the mixing time $t_0 = 0$.

[CAc.]	Exp.	t_1	t_{last}	t_{∞}	Δt_{acq}	Area [μm^2]
5.2	1	29 min, 15 s	1 h, 50 min	–	3.8 s	$(133.5 \times 71.1) = 9\,483$
	2	14 min, 47 s	1 h, 17 min	6 d, 21 h	13.6 s	$(71.1 \times 46.9) = 3\,333$
	3	13 min, 45 s	1 h, 39 min	–	3.8 s	$(129.1 \times 85.9) = 11\,086$
	4	12 min, 32 s	2 h, 22 min	20 d, 22 h	13.6 s	$(125.6 \times 73.3) = 9\,208$
	5	14 min, 33 s	3 h, 58 min	–	13.6 s	$(119.2 \times 66.7) = 7\,955$
0.0	1'	13 min, 50 s	1 h, 28 min	9 d, 2 h	13.6 s	$(132.3 \times 89.3) = 11\,824$
	2'	11 min, 25 s	2 h, 53 min	–	13.6 s	$(139.3 \times 93.4) = 13\,012$

123 2.4. X-ray tomography

124 Quantitative results obtained with SEM experiments at the surface of QX202C cells were compared
 125 with 3D reconstructions of microstructures of plaster set both within and outside the cells. Computed X-ray
 126 tomography (μ -CT) was performed on an EasyTom Nano (RX Solutions, Chavanod, France) with a voltage
 127 of 100 kV and a current intensity of 145 μA . The voxel size reached for the tomographies on set plaster
 128 within and outside the cell were 2.5 μm and 0.3 μm , respectively. The latter resolution enabled to performed
 129 cross sections in the volumes to compare surface and bulk information obtained from SEM and μ -CT with
 130 resolutions of 139.6 nm and 300 nm, respectively.

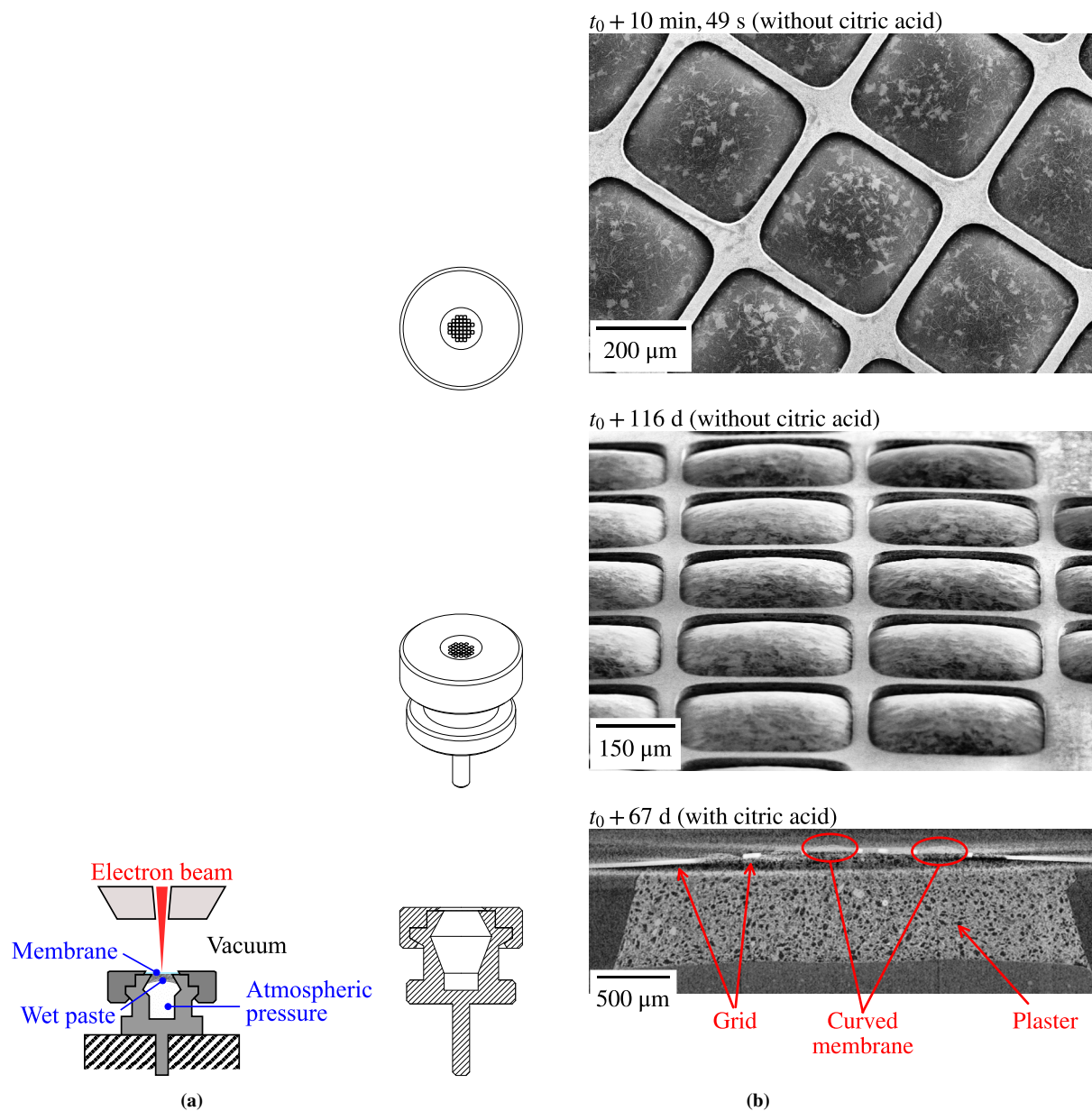


Figure 1: QuantomiX QX202C cell used to monitor DH crystals growth in liquid conditions in a SEM. (a) Scheme of the setup. (b) From top to bottom, observations of the cell with gypsum crystals: top and radial views obtained by SEM, cross section view obtained from a slice of a reconstructed volume with μ -CT (voxel size of 2.5 μm). Reproduced from doi: 10.6084/m9.figshare.23723673 © (2023) A. Fantou, CC BY 4.0 license <https://creativecommons.org/licenses/by/4.0/>.

131 3. Results

132 Figure 2 illustrates typical observations made during the setting of plaster without any retardant. In
 133 the first images, acquired around 13 min after mixing of powder and deionized water, many particles (in
 134 bright) are already observed, scattered in the liquid setting paste (in dark). The crystals present two types
 135 of morphologies: some are elongated, in the shape of thin needles of a few tens of micrometers long and

136 around 1 μm thick, while others form platelets, which often appear as flat and twinned crystals. The top
 137 view of the QX202C cell (fig. 2(a)) shows that the particle distribution is relatively homogeneous and similar
 138 among all windows of the grid: a focus at higher magnification can be made on a smaller zone to better
 139 describe the evolution of gypsum crystals with time (fig. 2(b)). While it seems that very few new crystals
 140 are formed after the first image (at t_1), most of them grow up to around 30 min, occupying a larger surface
 141 area than in the first image. No more significant evolution can be observed after around 30 min, and the
 142 same crystals can still be observed after a very long time ($t_\infty > 9$ days), when enough time is provided for
 143 the setting reaction to complete within the QX202C cell, kept out the microscope.

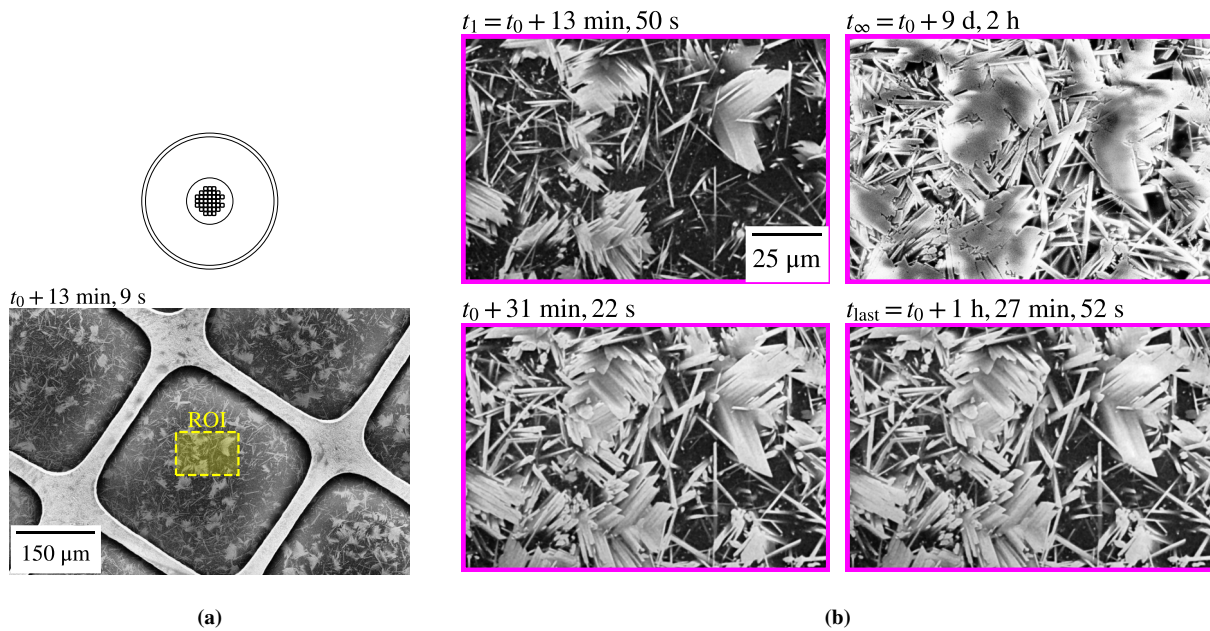


Figure 2: *In situ* micrographies of gypsum plaster crystals growing without citric acid in QX202C cell from experiment 1'. For experiment details, refer to table 1. (a) Overview of the grid and location of the region of interest (ROI) 13 min after t_0 and (b) Zoom in the ROI monitored at different times (including t_1 and t_{last} , times of first and last images, respectively, and t_∞ , collected many days after at the same location). The timelapse obtained from these micrographies can be found in [21]. Reproduced from doi: [10.6084/m9.figshare.23723673](https://doi.org/10.6084/m9.figshare.23723673) © (2023) A. Fantou, CC BY 4.0 license <https://creativecommons.org/licenses/by/4.0/>.

144 When the plaster is prepared with the 5.2 mM citric acid solution (fig. 3), no grown crystals are seen
 145 in the first images. Observed bright points (see for instance fig. 3(b), time t_1) may correspond to small HH
 146 particles not yet dissolved, to DH seeds or due to noise artifacts from the acquisition. Then, the growth of
 147 DH seeds can be monitored all along the experiment. The crystals grow for more than 2 h (fig. 3(b)). At
 148 this point, as for fig. 2, both platelets and acicular crystals can be observed, even though it seems that fewer
 149 twinned crystals can be observed than when the plaster is prepared without citric acid (fig. 2). The top view
 150 of the grid (fig. 3(a)) gives a more complete overview of the spatial distribution of gypsum crystals at the
 151 surface of the QX202C cell, at the beginning and after crystal growth (after ca. 13 min and 2h, respectively).
 152 Although the whole surface can be depicted as relatively homogeneous, denser (*i.e.*, brighter) zones can be
 153 observed among few places, where crystals are less densely packed (*i.e.*, darker areas).

154 The use of citric acid as a retardant enables to monitor better the early stages of the dissolution-
 155 precipitation reaction, as shown by comparing figs. 2 and 3. Experiments conducted with citric acid per-
 156 mitted to observe: (i) the initial presence of DH seeds followed by (ii) growth of DH crystals with time,

157 whereas only the step (ii) could be observed in experiments conducted without citric acid.

158 To quantify the evolution of the extent of the setting reaction with time, image analysis was performed
 159 on all stacks of micrographies obtained for each *in operando* experiment. To do so, HH and DH particles
 160 have to be identified to follow the dissolution of HH particles on the one hand and the precipitation of DH
 161 crystals on the other hand. The methodology developed in this work, based on segmentation of the imaged

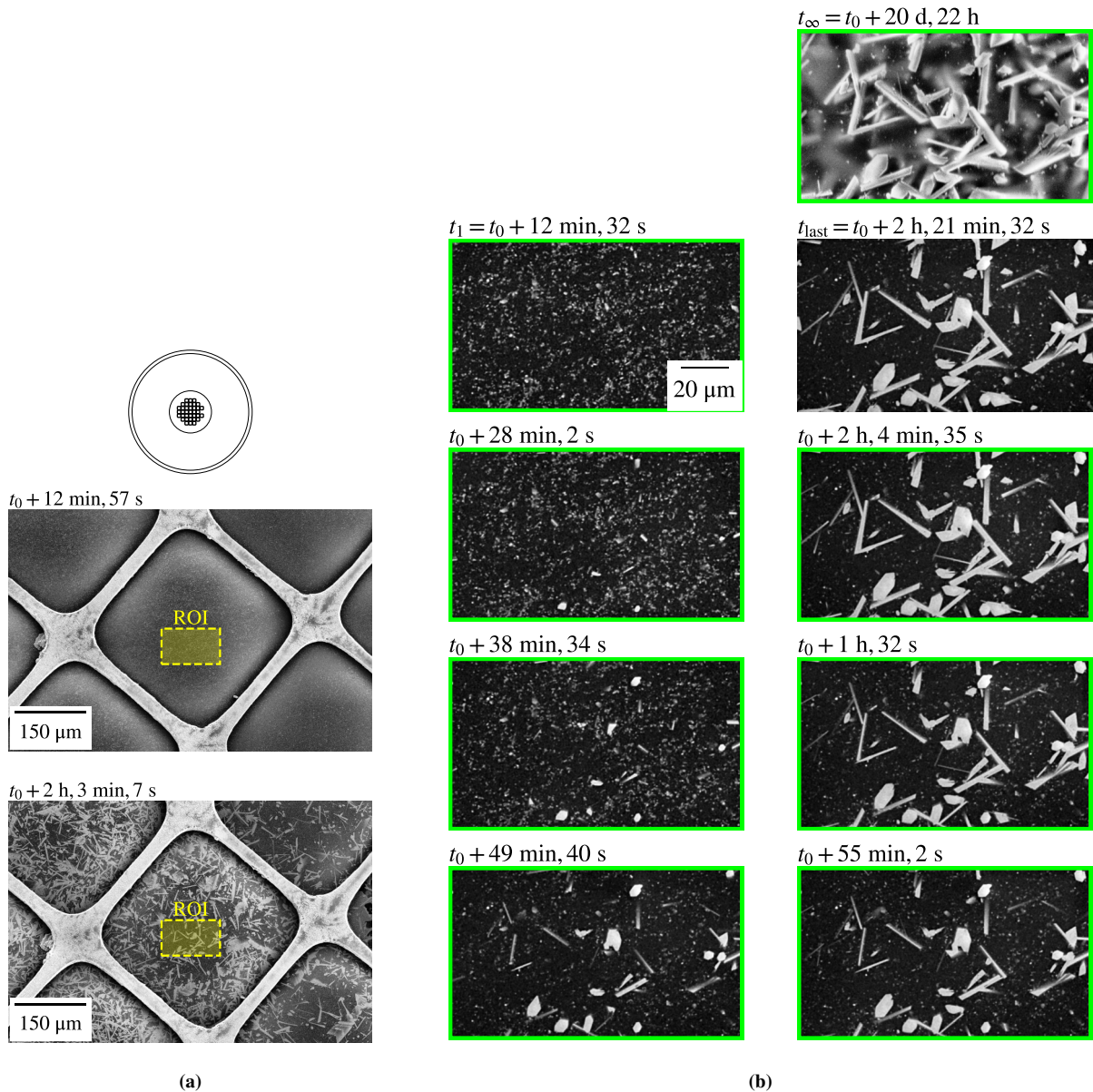


Figure 3: *In situ* micrographies of gypsum plaster crystals growing with citric acid in QX202C cell from experiment 4. For experiment details, refer to table 1. (a) Overview of the grid and location of the region of interest (ROI) 13 min (above) and 2h (below) after t_0 and (b) Zoom in the ROI monitored at different times (including t_1 and t_{last} , times of first and last images, respectively, and t_{∞} , collected many days after at the same location). A timelapse obtained from the micrographies of an equivalent experiment (experiment 1) can be found in [20]. Reproduced from doi: [10.6084/m9.figshare.23723673](https://doi.org/10.6084/m9.figshare.23723673) © (2023) A. Fantou, CC BY 4.0 license <https://creativecommons.org/licenses/by/4.0/>.

162 particles, is described below. Whereas it is easy to distinguish particles from the dark background based
163 on gray levels, HH and DH particles can be segmented neither on the basis of their relatively similar gray
164 levels, nor on the basis of their morphology. Especially in the first images (*i.e.*, for $t < \approx 30$ min), it is likely
165 that both HH and DH particles should be seen at this stage, but that DH is not crystallized enough yet to its
166 well-known shapes (needle, platelet).

167 To overcome this issue, segmentation of HH particles was performed based on the fact that their surface
168 area should decrease with reaction time as they dissolve during plaster setting (see eq. (1)). HH binary mask
169 was built for each analyzed raw image (named with a R), as illustrated in figs. S4 and S5 and detailed in sup-
170plementary information. To obtain DH masks, segmentation of DH crystals was performed by subtracting
171the HH masks from the corresponding binary images (named with A in figs. S4 and S5). This methodology
172can be validated by superimposing both HH and DH masks to their corresponding raw images. The fig. 4
173shows that the evolution of the HH (with red contours) and the DH (with blue contours) are consistent with
174micrographies at different stages of the setting reaction. Moreover, few HH particles are identified, and their
175dissolution process can not be monitored in these experiments. However, the fig. 4 highlights the interest of
176the method developed in this work to study the growth of DH crystals.

177 In the following, the extent of the setting reaction will be modelled by the surface fraction occupied by
178precipitating DH crystals ($\phi_{wp}(t)$). $\phi_{wp}(t)$ is computed at any time t from binary DH masks by dividing the
179number of white pixels ($n_{wp}(t)$) contained in the DH contours by the total number of pixels in the binary
180image (n_p), see eq. (2).

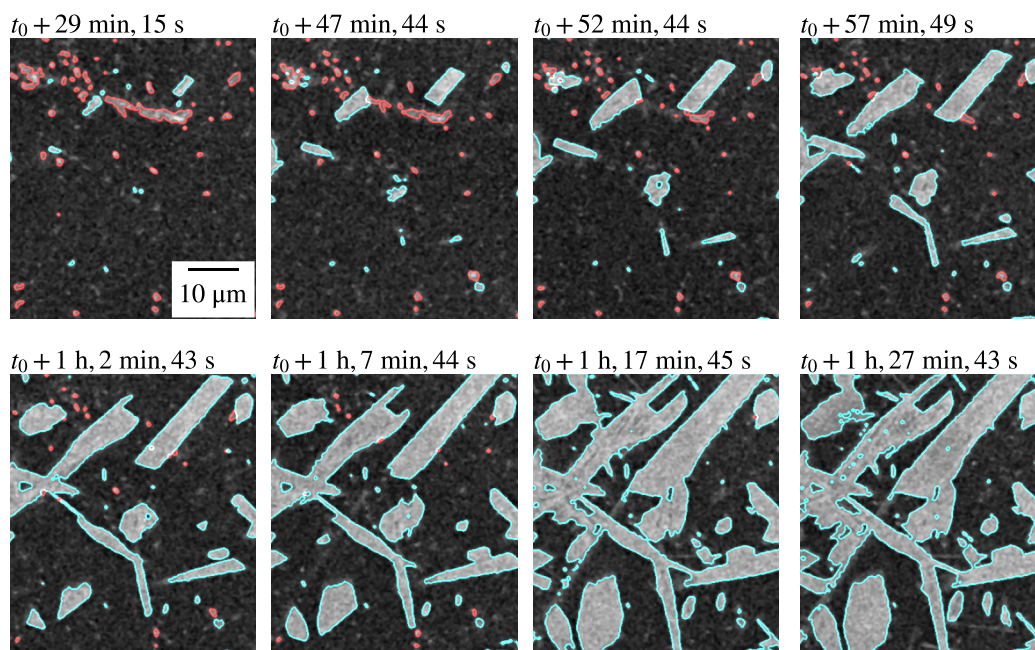


Figure 4: Image sequence of a fraction of the collected area from experiment 1 (5.2 mM citric acid). For experiment details, refer to table 1. After segmentation, HH and DH crystals can be correctly identified, respectively with red and blue outlines. Reproduced from doi: [10.6084/m9.figshare.23723673](https://doi.org/10.6084/m9.figshare.23723673) © (2023) A. Fantou, CC BY 4.0 license <https://creativecommons.org/licenses/by/4.0/>.

$$\phi_{\text{wp}}(t) = \frac{n_{\text{wp}}(t)}{n_{\text{wp}}(t) + n_{\text{bp}}(t)} = \frac{n_{\text{wp}}(t)}{n_{\text{p}}} \quad (2)$$

181 with ϕ_{wp} the surface fraction occupied by white pixels, n_{wp} , n_{bp} the number of white and black pixels,
 182 respectively, and n_{p} the total number of pixels.

183 In order to compare different experiments, with different surface crystal fractions in the observation
 184 area, a normalized surface density is computed as in eq. (3):

$$\overline{\phi_{\text{wp}}}(t) = \frac{\phi_{\text{wp}}(t)}{\max(\phi_{\text{wp}}(t))} \quad (3)$$

185 with $\overline{\phi_{\text{wp}}}$ the normalized surface fraction occupied by white pixels. The normalization is done respectively
 186 to the maximum value of ϕ_{wp} reached at plateau.

187 Figure 5 shows the evolution with time of the normalized surface fraction of DH crystals $\overline{\phi_{\text{wp}}}(t)$. For a
 188 better comparison of the qualitative observations with the quantitative evolution plotted in fig. 5, the magenta
 189 and green squares indicate the times at which micrographies displayed in figs. 2 and 3 were collected. As
 190 already observed in fig. 2, the DH crystals have already significantly grown at t_1 ($\overline{\phi_{\text{wp}}}(t_1) \approx 0.5$). Thus, it is
 191 impossible to monitor the beginning of the setting reaction without the use of any retardant. As expected,
 192 the presence of citric acid delays the beginning of the precipitation of DH crystals: red curves all show a
 193 time shift compared to the blue ones. The 5 red curves, computed from plaster setting in the presence of
 194 citric acid, highlight the reproducibility of the experiments in terms of times. Three regimes are observed
 195 and three characteristic times can be defined based on these regimes:

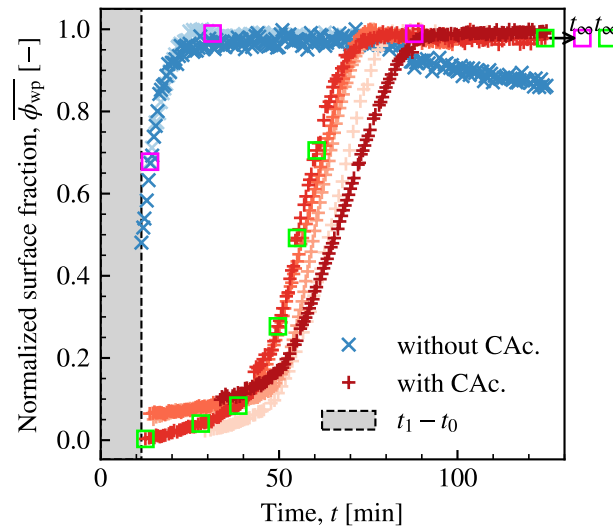


Figure 5: Evolution of the normalized surface fraction of white pixels $\overline{\phi_{\text{wp}}}$, between t_1 and t_{last} . Blue crosses and red plus markers represent experiments conducted without ($N = 2$, pale and dark blue) and with ($N = 5$, different shades of red) citric acid (CAC.), respectively. For experiment details, refer to table 1. $\overline{\phi_{\text{wp}}}(t_{\infty})$ was not computed as micrographies at t_{∞} were recorded with different conditions, several days after t_{last} . The magenta and green squares highlight times at which were collected the micrographies displayed in figs. 2(b) and 3(b), respectively. The gray area represents the dead-time between the mixing time t_0 and the first acquisition t_1 . The evolution of the (non-normalized) surface fraction ϕ_{wp} is displayed in fig. S1. Reproduced from doi: 10.6084/m9.figshare.23723673 © (2023) A. Fantou, CC BY 4.0 license <https://creativecommons.org/licenses/by/4.0/>.

- 196 • during the induction period, the solid fraction of DH crystals slowly evolved up to the time t_2 (end of
197 the induction regime, see fig. S2 for the definition of characteristic times), when the increase of $\overline{\phi_{wp}}(t)$
198 accelerated;
- 199 • time t_3 was defined as the inflexion point in the acceleration regime (see fig. S2);
- 200 • after time t_4 , the termination regime started as $\overline{\phi_{wp}}(t)$ reached a plateau ($\overline{\phi_{wp}}(t) \rightarrow 1$): the growth of
201 DH crystals slowed down until no more significant change could be detected. Only in the experiment
202 2' (in dark blue, without citric acid), $\overline{\phi_{wp}}(t)$ decreased $\approx 50 \text{ min}$ after t_4 . This was probably due to an
203 artifact arising from a shift in contrast in the images acquired after more than 80 min.

204 The non-normalized surface fraction of DH crystals, $\phi_{wp}(t)$, is also plotted in fig. S1. In addition to
205 what was already seen from the evolution of the normalized surface fraction $\overline{\phi_{wp}}(t)$, it also highlights the
206 dispersion of the final surface fraction of DH crystals between the different experiments. Indeed, $\phi_{wp}(t_{\text{last}})$
207 ranged from $\approx 20_{\text{surf}}\%$ to $\approx 75_{\text{surf}}\%$. This is explained by the relatively small surface areas monitored
208 during the *in operando* acquisitions and by the variation of local densities in terms of crystals from one
209 region to one another (see for example fig. 3(a)).

210 To provide quantitative data on the kinetics of the setting reaction, the growth rate of DH crystals $\frac{\partial\phi_{wp}}{\partial t}$
211 was measured at time t_3 , *i.e.*, the inflexion point in the acceleration regime (see fig. S2 for the definition of
212 characteristic times). Table 2 summarizes this DH growth rate $\frac{\partial\phi_{wp}}{\partial t}(t_3)$, as well as all other quantitative data
213 obtained from experiments performed with citric acid. Once again, the reproducibility of the experiments
214 in terms of times is highlighted by the relatively small dispersions between the 5 values of t_2 , t_3 and t_4 ,
215 respectively. When the induction regime ends at t_2 , DH crystals occupied $\approx \frac{1}{5}$ of their surface area at the end
216 of the setting reaction ($\overline{\phi_{wp}}(t_2) \approx 0.20$). For all of the 5 experiments, the inflexion point of the acceleration
217 regime was reached when DH crystals had grown to $\approx \frac{1}{2}$ of their final surface area ($\overline{\phi_{wp}}(t_3) \approx 0.55$).
218 Regarding the kinetics of the DH growth, $\frac{\partial\phi_{wp}}{\partial t}(t_3)$ ranges from $1.6\% \text{ min}^{-1}$ to $3.1\% \text{ min}^{-1}$.

219 Furthermore, a quantitative analysis of gypsum crystals growth can be performed. To display the evo-
220 lution with time of DH crystals, a superimposition of all their successive contours along one experiment is

Table 2: Characteristic times t_2 (end of induction regime (I)), t_3 (inflexion point in the acceleration regime (II)) and t_4 (start of termination regime (III)) determined from curves displayed in fig. 5 for all experiments with citric acid. Mean values and standard-deviations (std) were computed for each parameter ($N = 5$). For experiment numbers and details, refer to table 1. These characteristic times were computed using the second derivative of $\overline{\phi_{wp}}$: $\frac{\partial^2(\overline{\phi_{wp}})}{\partial t^2}$, as illustrated in fig. S2. ϕ_{wp} and $\overline{\phi_{wp}}$ are the surface fractions of white pixels at given times, non-normalized and normalized with respect to the maximum value of ϕ_{wp} reached at plateau, respectively.

Exp.	(I)			(II)				(III)		
	t_2 [min]	$\phi_{wp}(t_2)$ [%]	$\overline{\phi_{wp}}(t_2)$ [-]	t_3 [min]	$\phi_{wp}(t_3)$ [%]	$\overline{\phi_{wp}}(t_3)$ [-]	$\frac{\partial(\phi_{wp})}{\partial t}(t_3)$ [% min ⁻¹]	t_4 [min]	$\phi_{wp}(t_4)$ [%]	$\overline{\phi_{wp}}(t_4)$ [-]
1	52.59	9.01	0.17	65.47	29.88	0.55	1.77	77.55	50.50	0.93
2	52.42	7.28	0.20	60.44	18.72	0.52	2.01	76.48	35.65	1.00
3	45.98	8.48	0.18	58.70	26.47	0.56	2.88	71.43	44.48	0.95
4	46.15	4.42	0.21	56.23	11.59	0.54	1.63	67.44	19.34	0.90
5	53.66	15.83	0.23	68.28	39.58	0.57	3.09	83.82	64.12	0.92
Mean	50.16	9.00	0.20	61.83	25.25	0.55	2.28	75.34	42.82	0.94
Std	3.37	3.76	0.02	4.43	9.57	0.02	0.59	5.58	14.95	0.03

221 shown in fig. 6(a) (in black) and in fig. 6(b) (colors changing with time t). These images combine infor-
 222 mation on crystal shape, growth direction and kinetics. As the time step between two acquired images is
 223 constant in a sequence, large distances between successive contours are indicative of fast crystal growth.

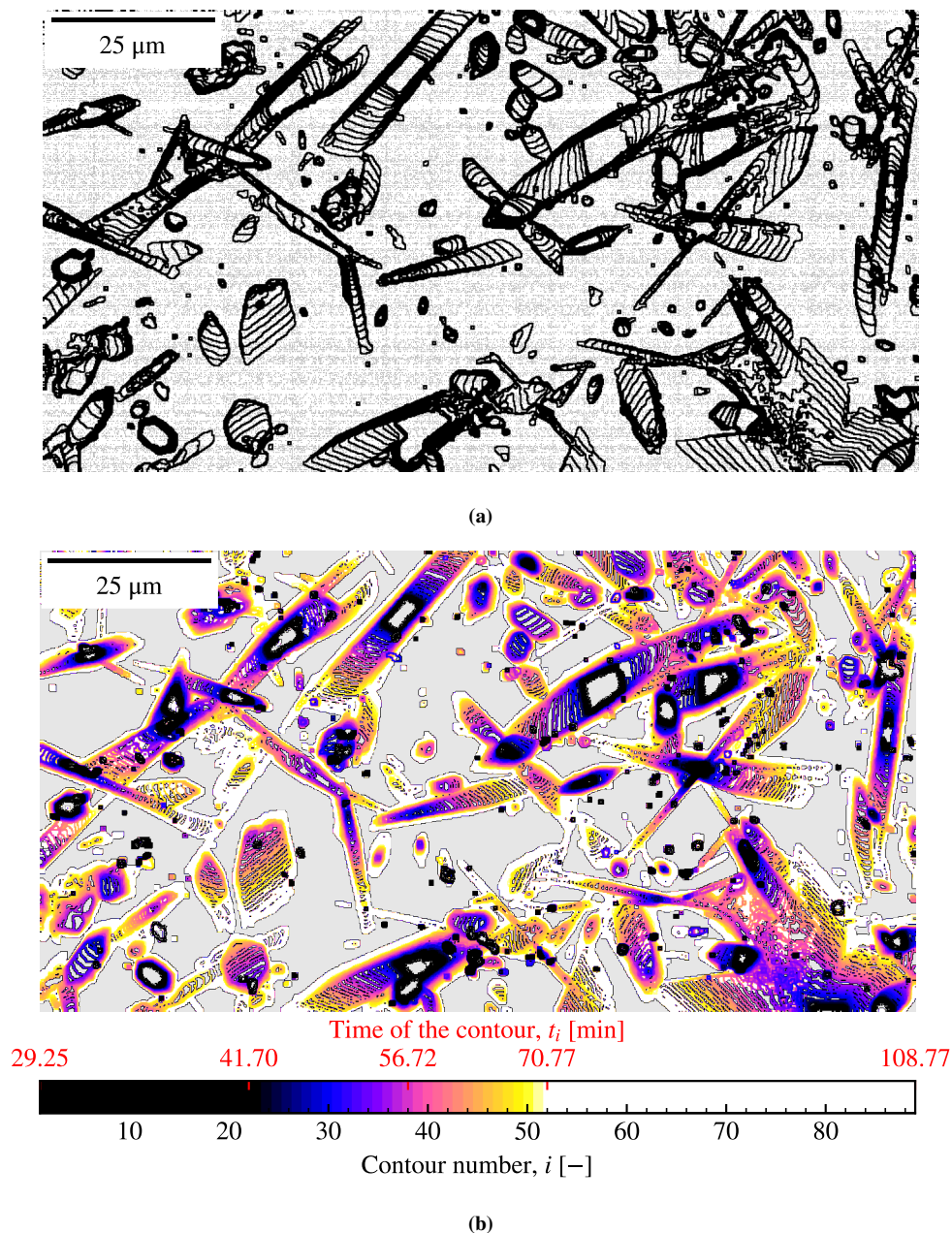


Figure 6: Evolution of DH crystals contours with time, from experiment 1 with citric acid. For experiment details, refer to table 1. (a) focus on the beginning of the crystal growth: superimposition of DH contours from 42 min to 70 min (constant time step between 2 successive contours, $t_{\text{step}} = 2$ min); (b) superimposition of all the DH contours up to t_{last} . Time scale is displayed by the colors gradation and by the increase of contour number i (constant time step between 2 successive contours, $t_{\text{step}} = 1$ min). Reproduced from doi: [10.6084/m9.figshare.23723673](https://doi.org/10.6084/m9.figshare.23723673) © (2023) A. Fantou, CC BY 4.0 license <https://creativecommons.org/licenses/by/4.0/>.

224 On the contrary, close lines or contours superimposed on each other mean that the growth rate was low or
225 even negligible, as is generally the case below t_2 .

226 From fig. 6(b), DH crystals can be classified into at least four categories according to their growth
227 modes: longitudinal growth or isotropic mode, growth from an apex, or from a side of the crystal. These
228 different growth modes may be explained by different crystal orientations.

229 To further investigate this issue, six isolated DH crystals with longitudinal growth mode have been
230 chosen from experiments 1 and 2 (refer to table 1 for experiment details). Their length and width have been
231 measured over about 100 images collected during each experiment. The evolution of the crystal dimensions
232 with time is plotted in figs. 7(a) and 7(b). Interestingly, their evolution is similar and three growth regimes
233 can be identified. In regime (I), the length increased slowly when compared to regime (II), during which the
234 longitudinal growth rate reached a typical value of 18 nm/s. On the contrary, the crystal width increased
235 linearly with time, with no change in velocity between regimes (I) and (II) (fig. 7(b)). In regime (III), the
236 growth stopped, most probably due to crystals coming into contact with others, as supported by fig. 6(b).
237 It is also noteworthy that the transitions from regimes (I) to (II), and from regimes (II) to (III), occurred at
238 approximately constant times, respectively $t \approx 50$ min and $t \approx 75$ min to 80 min.

239 Figure 7(c) displays the shape of one of the six isolated DH crystals, at times $t = 35$ min (regime
240 (I)) and $t = 1$ h, 4 min (regime (II)). The change in the angles measured directly on the DH contours (see
241 fig. 7(c)) was concomitant with the transition from regimes (I) to (II), which appears to be indicative of
242 crystal structure changes. To identify the crystallographic planes in regime (II), data from the literature
243 describing DH crystal facets at equilibrium [22, 23] can be used. We also have to take into account the fact
244 that the DH contours are obtained from top-views of the crystals. Hence, the edges of the contours do not
245 necessarily correspond to real facets but may actually correspond to the projection of facets onto the plane
246 of the QuantomiX cell membrane. With this in mind, DH crystals were modeled with the software Vesta
247 [24] using the $C 2/c$ monoclinic structure from [25] (see figs. 7(d) and 7(e)).

248 An excellent agreement is found between the experimental contours (fig. 7(c)) and the crystal top views
249 (fig. 7(d)), with long and short sides defined from the intersection of $\{120\}$ and $\{111\}$ facets, respectively. It
250 is noteworthy that the $\{010\}$ facet was the one in contact with the cell membrane. This is consistent with the
251 fact that the cell membrane is hydrophilic and that water molecules in DH crystals are organized in planes
252 corresponding to $\{020\}$ facets.

253 Based on the indexation for the crystal structure in regime (II), an indexation can be proposed for the
254 DH crystal in regime (I), with the same zone axis. Obviously, the longest sides of the crystal already
255 corresponded to the intersection of $\{120\}$ facets. However, for the shortest sides, no agreement could be
256 found between the planes present in the crystal at equilibrium and the experimental contour (fig. 7(c)).
257 From the value of the angles, we hypothesize that the shortest sides corresponded to the projection of $\{445\}$
258 or $\{455\}$ planes. As these planes are not thermodynamically stable but very similar to $\{111\}$ obtained at
259 equilibrium, this could explain the transition from regime (I) to regime (II).

260 Similarly, the indexation of several crystals, growing either longitudinally, or isotropically, or from a
261 side, is summarized in fig. 8. For each crystal, the indexation at the end of the setting reaction contains
262 only the equilibrium facets described in the literature [22, 23]. Misorientations between the models and the
263 experimental angles of the DH contours remained below 14° (mostly below 10°), comforting the hypotheses
264 made for the indexation of the facets.

265 Finally, growth rates were determined for each specific crystallographic plane identified previously, and
266 for all growth regimes, using the evolution of DH contours with time. To account for the fact that not all
267 growing facets were parallel to the cell membrane, the measured apparent growth rates have been corrected
268 to obtain average growth rates in the perpendicular direction to the considered facets (see fig. 8).

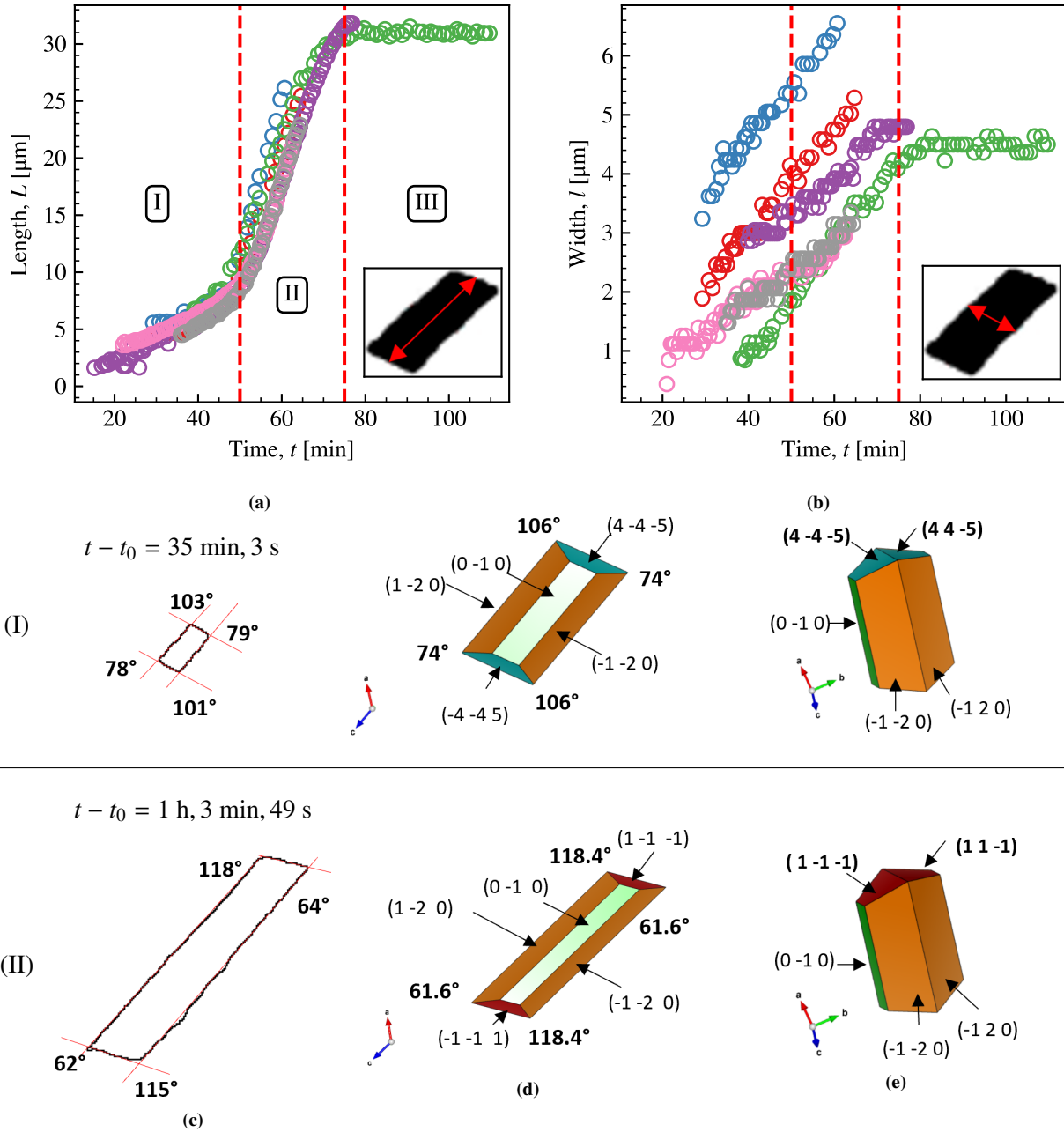


Figure 7: Evolution with time of 6 DH crystals following a longitudinal growth; crystals from experiments 1 to 5, with citric acid. (a) Evolution of the length of DH crystal versus time $N = 6$. (b) Evolution of the width of DH crystal versus time $N = 6$. (c) Experimental measurements of angles of a typical DH crystal. (d) 2D and (e) 3D views (built with Vesta software [24]) of the crystallography of a typical DH crystal. Crystal modelization was based on the experimental angles and 2D morphology and on the equilibrium facets. Reproduced from doi: [10.6084/m9.figshare.23723673](https://doi.org/10.6084/m9.figshare.23723673) © (2023) A. Fantou, CC BY 4.0 license <https://creativecommons.org/licenses/by/4.0/>.

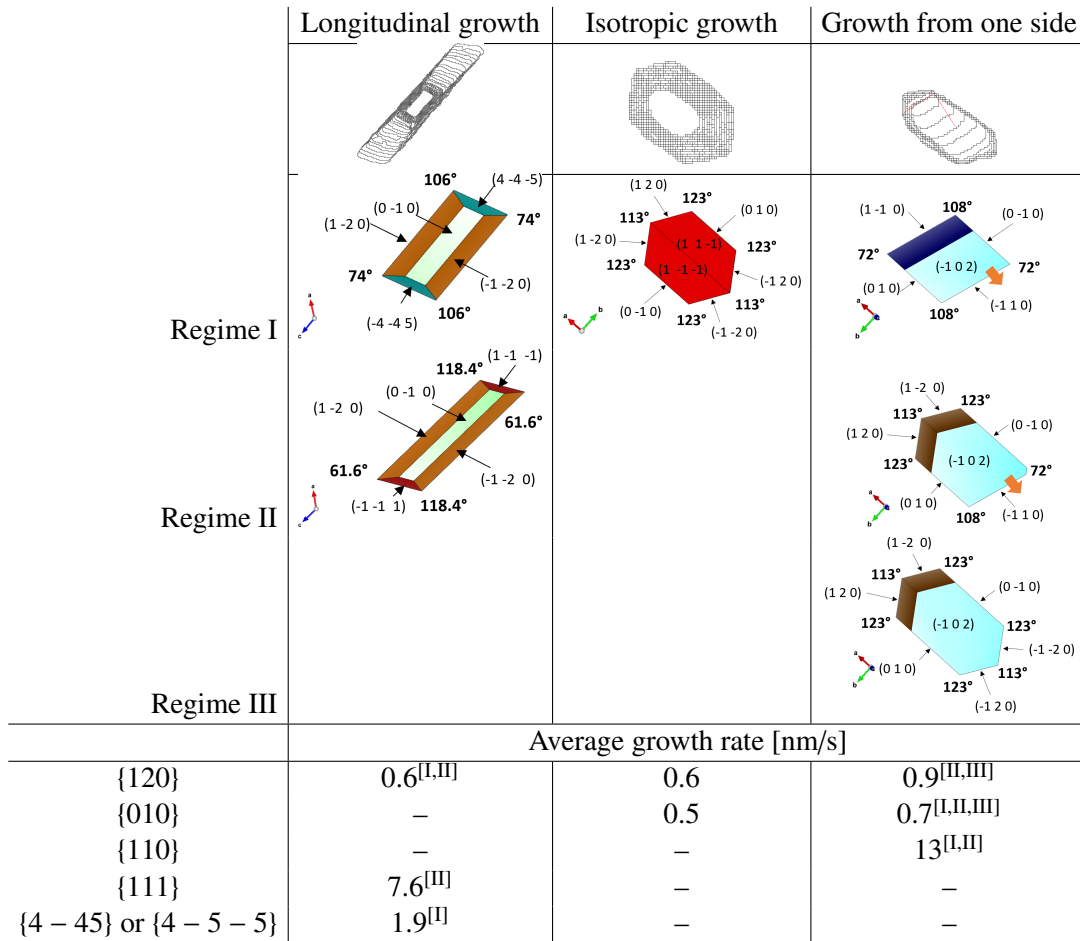


Figure 8: Typical DH crystal indexation for 3 different types of crystal growth (longitudinal, isotropic or growth from one side) and measured average growth rates of crystal planes. In this work, DH crystals were studied from experiments 1 to 5, with citric acid. Reproduced from doi: [10.6084/m9.figshare.23723673](https://doi.org/10.6084/m9.figshare.23723673) © (2023) A. Fantou, CC BY 4.0 license <https://creativecommons.org/licenses/by/4.0/>.

4. Discussion

The aims of this study were:

- to implement a robust methodology for the *in operando* monitoring at micro-scale of the evolution of the microstructure of hydraulic binders during their setting process and with a L/S used in application;
- to characterize the setting of gypsum plaster through the observations of the dissolution of HH particles and the precipitation of DH crystals;
- to quantify the kinetics of the dissolution and precipitation reactions from image analysis, with sufficient temporal and spatial resolutions;
- to better understand the mechanisms of nucleation and growth of individual DH crystals in relation to their crystallography.

The use of encapsulating cells enabled to investigate the evolution of setting pastes in conditions close to the ones in application (*e.g.* pressure, temperature, liquid to solid mass ratio) and with a very good spatial resolution (pixel size of ≈ 100 nm). The protocol used in this work enabled a precise control of the L/S ratio and thus of the supersaturation reached in the paste when HH particles dissolve. Both these parameters have a strong influence on the kinetics of the reactions and on the development of the microstructure one aims to follow. Indeed, in such concentrated pastes, growing DH crystals eventually contact or/and entangle with each others (see fig. 6), which may influence their later growing mechanism.

To observe setting processes in SEM without the use of such sealed cells, it would have been necessary to stop the setting process, for instance by immersion of the setting paste in a solvent such as ethanol [3, 26, 27]. This would enable SEM observation with a good resolution but without the possibility to follow the evolution of the microstructure of a same sample, and with a risk of affecting it due to the quenching protocol. The monitoring of dissolution and precipitation in liquid-phase STEM-in-SEM (Scanning Transmission Electron Microscopy) is also possible, but it requires very dilute conditions and is thus more distant from the real conditions of application of hydraulic binders [28]. Finally, the method developed in this work appears to be complementary to other techniques:

- X-ray tomography with concentrated pastes representative of the application and enabling a characterization of the bulk of the setting paste with 3D measurements [16, 17], but with a degraded spatial resolution;
- AFM to follow the dissolution/precipitation in very diluted suspensions, with a very good resolution at the scale of single crystals, not entangled and without control of the amount of liquid [11–15].

Because of the dead-time needed for the sample preparation, and probably also due to the design of the environmental cell, few HH particles were identified (represented with red outlines in fig. 4). Thus, it was not possible to quantify the kinetics of their dissolution. On the bright side, the method developed in this work proved to be very efficient and with sufficient time and spatial resolutions to study in detail the growth of relatively small DH crystals evolving quickly. This dynamic process was quantified by image analysis both at a microscale (ROI containing more than a hundred DH crystals, fig. 5) and at the scale of individual DH crystals (fig. 7). Kinetics measured at the microscale provided mean values, less sensitive to heterogeneities in particles, while follow-up of individual crystals permitted to relate their growth regimes to changes in crystallography. This is especially interesting as data is lacking on precipitation mechanisms and discrepancies still exist in the literature. For instance, while in some studies DH crystals are thought to nucleate on dissolving HH particles [8], others advocate for a nucleation of DH crystals nearby HH particles, in confined supersaturation regions [16–18].

4.1. Representativeness of the *in operando* observations

Even if the methodology was carefully designed in order to minimize any effect on the observed transformation and kinetics, the representativeness of results obtained from these *in operando* setting experiments should be addressed before extrapolating these results to gypsum plasters used in applications. First, due to the protocol, t_1 could not be smaller than 10 to 15 min. Hence, it was necessary to use citric acid as a setting retardant to be able to monitor the reaction since its beginning. Indeed, the fig. 5 shows that, without any retardant, the reaction was already too advanced at $t = t_1$. However, it should be taken into account that the addition of citric acid may influence the growth regimes and rates of DH crystals [29–31].

Second, the experiments were carried on a small volume of paste (7 μL in the Quantomix QX202C). Due to the exothermic character of the setting reaction, its kinetics could be slowed down compared to bigger volumes of setting plaster [8].

With the methodology developed here, the reaction kinetics was repeatable (see fig. 5). However, from one experiment to another one, the surface fraction reached at t_{last} varied from 20 $\text{surf}\%$ to 75 $\text{surf}\%$ (see fig. S1). Indeed, the local crystal density was observed to be heterogeneous depending on the chosen region of interest (ROI) on a same cell (see fig. 3(a)). We believe that it may be due to variations in the sample preparation: the concentration of the two drops successively pipetted within a larger volume of setting paste and their deposit on the membrane without the possibility to further homogenize it.

In the following, to better discuss its representativeness, the experimental surface fraction reached at t_{last} within the setup was compared to the expected surface fraction of solid, computed from the setting reaction (see eq. (1)), and to an experimental surface fraction of solid measured from X-ray tomography acquisitions on *ex situ* set samples. From eq. (1), if one considers the volume fraction of void (*i.e.*, pores) to arise from the excess water and the volume shrinkage (due to the mass density difference between HH and DH), it comes that the volume fraction of solid (*i.e.*, crystals) once the reaction is completed can be expressed as in eq. (4).

$$\Phi_{\text{solid}}\left(\frac{L}{S}\right) = \frac{\frac{M_{\text{DH}}}{M_{\text{HH}}} \times \frac{\rho_{\text{H}_2\text{O}}}{\rho_{\text{DH}}}}{\left[\frac{L}{S}\right] + \frac{\rho_{\text{H}_2\text{O}}}{\rho_{\text{HH}}}} = \frac{0.51}{0.60 + 0.38} = 0.52 \quad (4)$$

with Φ_{solid} the volume fraction of solid (*i.e.*, crystals), M_{DH} , M_{HH} the molar masses of calcium sulfate dihydrate and hemihydrate, respectively (*i.e.*, bassanite and gypsum) and $\rho_{\text{H}_2\text{O}}$, ρ_{DH} the mass densities of water and calcium sulfate dihydrate, respectively. With the used L/S ratio, it comes that the expected final volume fraction of solid is $\Phi_{\text{solid}} = 52 \text{ vol}\%$. If the density of the material is homogeneous and isotropic, then the surface fraction of solid should be strictly equal to the volume fraction of solid. Thus, $\phi_{\text{sol}} = \Phi_{\text{sol}} = 52 \text{ surf}\%$.

Complementary, X-ray tomography acquisitions were performed, focusing on the core of samples of gypsum plaster set *ex situ* (*i.e.*, out of any cell) with citric acid. From a same 3D reconstruction, eleven 2D cross sections of surface areas equivalent to the ones analyzed in SEM experiments, *i.e.*, $(130 \times 70) \mu\text{m}^2$ were selected randomly. The fig. S3 exhibits the results of the analysis of the surface fraction of solid of these cross sections. It is shown that on 11 cross sections, the surface fraction of solid varied from $\phi_{\text{sol}} = 51 \text{ surf}\%$ to $\phi_{\text{sol}} = 73 \text{ surf}\%$, with a mean value of $\phi_{\text{sol}} = (60.6 \pm 8.2) \text{ surf}\%$. It is worth noting that the total volume fraction of solid in the whole sample was determined to be 59 $\text{vol}\%$. With these results, we can reasonably consider that experiments in which the final surface fraction of solid was within 50 $\text{surf}\%$ and 60 $\text{surf}\%$ (fig. S1) were representative of real conditions. Moreover, this does not seem to be critical since the aim of this study was not to quantitatively measure the volume fraction of gypsum plaster, which is more conventionally characterized by X-ray tomography or mercury intrusion porosimetry.

4.2. Potential artifacts induced by the developed methodology

Due to the presence of the electron beam, irradiation damage in the setting paste may be expected and cause artifacts as it is known to induce water radiolysis [32, 33]. Irradiation damage may generate reactive species, such as aqueous electrons e_{aq}^- , or protons and other species as H^+ , OH^- , $\text{H}\cdot$, $\text{OH}\cdot$. These species can change the solution pH, and thus influence the reaction mechanisms characterized in this paper [8]. In order to limit its influence, the electron beam was blanked between each acquisitions (taken every 30 s then every 1 min). In these conditions, no obvious signs of irradiation damage were visible in the monitored region. Moreover, the comparison with non-irradiated regions of the encapsulating cell, observed either during or after the setting reaction, has shown no significant difference, which could be allocated to the electron beam (*e.g.*, similar crystals morphology and density).

Even with a quite large depth of field, the crystals that were imaged were the ones located at the surface of the cell, close to the membrane. Monte Carlo simulations (*cf.* table S1) suggested that the probed depth was about 200 nm below the membrane. Therefore, DH crystals nucleating below the probed depth would not be detected from the beginning and may become visible only when they grow up sufficiently for their extremity to become close enough to the membrane, being then counted as new nuclei. However, it did not seem to be the case for many DH crystals (fig. 4).

This is consistent with the expected preferential orientation of DH crystals nucleating and growing close to the surface, owing to the presence of the cell membrane and to its hydrophilic nature. Even though the cell membrane was not rigid (see its curvature in fig. 1), its presence may have affected the orientation of the DH crystals, with an expected preferential orientation of {010} facets parallel to the membrane.

All this highlights the importance of combining characterization techniques, at different scales, to draw the whole picture and provide explanations on the setting mechanisms.

4.3. Crystal indexation and atomic plane growth rates

These *in operando* SEM experiments provided information on the crystallization stages of gypsum and their kinetics. Indeed, in the case of retarded samples, for which the induction period could be observed, the normalized surface fraction of gypsum exhibited a three-stage evolution with time, describing a sigmoid curve (see fig. 5). The first regime corresponded to slow germination of the crystals and lasted until $t \approx 50$ min. Then, in a second regime, these crystals were observed to grow more rapidly. The rate of the solid fraction in the different ROI, given by the slopes of the non-normalized curves (table 2) ranged from 1.6 $\text{surf}\%/\text{min}$ to 3.1 $\text{surf}\%/\text{min}$. At a certain point, the crystal growth slowed down and the surface fraction of gypsum reached a plateau at $t \approx 75$ min to 80 min corresponding to the third regime.

These variations in growth rates at the microscopic level are also evidenced by electron microscopy images (fig. 6(b)). Regime (I) corresponds to crystals with black outlines: contours are close to each others, indicating that the crystals evolved slowly. Then, during the regime (II), the crystals grew rapidly (blue, pink then yellow contours), before reaching a stable size, corresponding in most cases to contacts between each crystal. Interestingly, the regimes corresponded exactly to the ones defined at the macroscopic level. In fig. 6, we can see that the majority of the crystals visualised in 2D are longitudinally growing needles. Other crystals, a minority in number and surface area, have an isotropic growth, or grew from an edge or an apex. It can therefore be assumed that the rate of surface densification was directly related to the rate of growth of longitudinal crystals. It can also be considered that most of the crystals visible on the images correspond to needles seen under different orientations.

The changes in crystal growth rates from regime (I) to regime (II) was associated to crystalline modifications (fig. 7). Indexations of the crystal facets have been suggested, considering that the shape of the crystals after long reaction times (hence in regime (II)) corresponded to their shape at equilibrium [22, 23]. The indexation of {120} and {010} facets was straightforward as they are well known in the literature. In regime

397 (I), more exotic {445} or {455} planes have been evidenced. The presence of unstable planes in regime (I)
398 could be evidenced here thanks to the developed methodology combining *in operando* and nanometer-scale
399 spatial resolution. The transformation from {445} or {455} planes to {111} planes, with almost similar Miller
400 indices, supports the proposed indexation.

401 As far as the growth rates of crystallographic planes are concerned (fig. 8), it is difficult to draw a
402 comparison with values given in the literature, in which growth rates were usually measured using AFM
403 on diluted solutions. It is indeed known that the growth rates depend on solution supersaturation [9], and,
404 to our knowledge, this is the first *in operando* study, in which growth rates of DH crystalline planes was
405 measured in concentrated plaster pastes (L/S representative of industrial applications). For instance, {010}
406 facets were sometimes qualified as stable, with a slow growth [10] and as very reactive in other studies [11].
407 Such discrepancies may arise from different experimental conditions (especially L/S ratios). However, it
408 is generally accepted that the {120} facets of DH crystals are stable, whereas the {111} are less stable and
409 grow fast [10, 11]. Also, the growth rate of {010} facets is known to be reduced in the presence of Na-
410 citrate retardant [9]. The main advantages of our experiment lies in the fact that this non-destructive method
411 enables the *in operando* follow-up of a same sample throughout the whole setting process with a liquid
412 phase representative from the real plaster setting (L/S , excepted for the presence of retardant). Being able
413 to quantify the DH growth rates in conditions representative of application is particularly important as it is
414 known to govern the development of the microstructure during setting, the entanglement of DH crystals and
415 of intercrystalline bonds, which in turn play a critical role in the mechanical properties of the final material.

416 5. Conclusions

417 The *in operando* SEM study of the precipitation of gypsum crystals from the dissolution of calcium
418 sulfate hemihydrate particles was made possible in standard conditions by the use of a specific encapsulating
419 cell without and with citric acid (5.2 mM). The size of the observed region was typically $(130 \times 70) \mu\text{m}^2$ with
420 a resolution of 139.6 nm, which enabled to monitor the evolution of dozens of crystals. Image analysis based
421 on segmentation provided quantitative results on the growth of crystals at global (*i.e.*, region of observation)
422 and local (*i.e.*, single crystal) scales. Different crystal growth regimes were observed (*e.g.*, longitudinal,
423 isotropic, from a side) due to different crystal orientations. An indexation of single crystal facets based
424 on crystallography and literature results was proposed and the crystal growth rates were measured for 5
425 crystallographic planes. It was found that:

- 426 • the {111} and {110} planes were the fastest growing planes, with growth rates of ≈ 8 nm/s and \approx
427 13 nm/s, respectively;
- 428 • the {120} and {010} planes were the most stable and grew slowly at similar rates of ≈ 0.7 nm/s;
- 429 • the {4 – 45} or {4 – 5 – 5} were identified as unstable planes, growing at ≈ 2 nm/s. Such unstable
430 planes transformed into {111} during the setting, causing a change in the growth regime and kinetics.

431 Kinetics variations, observed locally at the scale of individual DH crystals, were totally consistent with mean
432 variations measured at the microscopic scale, displaying a sigmoid evolution in three stages (induction,
433 acceleration and termination regimes).

434 Competing interests

435 The authors declare none.

436 Acknowledgements

437 This work was supported as part of SUN7 project (StrUcturiNg and SETting processes of mineral
438 cements for bone repair) funded by the French ANR [ANR-19-CE08-0008]. The authors would like to
439 thank Thierry Douillard for his support on SEM techniques, Jérôme Adrien for his support on the X-ray
440 tomography acquisition and the mechanical workshop of Mateis Laboratory for the manufacturing of the
441 QuantomiX cell holders.

442 Data availability statement

443 Timelapses gathering raw images (before image analysis) of representative experiments are available
444 from <https://hal.science/hal-04168810> and <https://hal.science/hal-04168814>. Other raw
445 and processed data required to reproduce these findings cannot be shared at this time as the data also forms
446 part of an ongoing study.

447 References

- 448 [1] A. Packter, The precipitation of calcium sulphate dihydrate from aqueous solution, *Journal of Crystal Growth* 21 (1974)
449 004–009. doi:10.1016/0022-0248(74)90004-9.
- 450 [2] M. E. Tadros, I. Mayes, Linear growth rates of calcium sulfate dihydrate crystals in the presence of additives, *Journal of*
451 *Colloid and Interface Science* 72 (1979) 245–254. doi:10.1016/0021-9797(79)90106-1.
- 452 [3] A. J. Lewry, J. Williamson, The setting of gypsum plaster – Part II The development of microstructure and strength, *Journal*
453 *of Materials Science* 29 (1994) 5524–5528. doi:10.1007/BF00349943.
- 454 [4] U. Tritschler, M. Kellermeier, C. Debus, A. Kempter, H. Cölfen, A simple strategy for the synthesis of well-defined bassanite
455 nanorods, *CrystEngComm* 17 (2015) 3772–3776. doi:10.1039/c5ce00519a.
- 456 [5] A. E. S. Van Driessche, T. M. Stawski, L. G. Benning, M. Kellermeier, Calcium Sulfate Precipitation Throughout Its Phase
457 Diagram, Springer International Publishing, Cham, 2017, pp. 227–256. doi:10.1007/978-3-319-45669-0_12.
- 458 [6] A. Katz, A. Bentur, K. Kovler, A novel system for in-situ observations of early hydration reactions in wet conditions in
459 conventional SEM, *Cement and Concrete Research* 37 (2007) 32–37. doi:10.1016/j.cemconres.2006.09.014.
- 460 [7] A. E. S. Van Driessche, L. G. Benning, J. D. Rodriguez-Blanco, M. Ossorio, P. Bots, J. M. García-Ruiz, The role and
461 implications of bassanite as a stable precursor phase to gypsum precipitation, *Science* 336 (2012) 69–72. URL: <https://science.sciencemag.org/content/336/6077/69>. doi:10.1126/science.1215648.
- 462
- 463 [8] M. Ilett, H. M. Freeman, Z. Aslam, J. M. Galloway, D. P. Klebl, S. P. Muench, I. J. McPherson, O. Cespedes, Y. Y. Kim,
464 F. C. Meldrum, S. R. Yeandel, C. L. Freeman, J. H. Harding, R. M. D. Brydson, Evaluation of correlated studies using liquid
465 cell and cryo-transmission electron microscopy: Hydration of calcium sulphate and the phase transformation pathways of
466 bassanite to gypsum, *Journal of Microscopy* n/a (2022). doi:10.1111/jmi.13102.
- 467 [9] D. Bosbach, M. F. Hochella, Gypsum growth in the presence of growth inhibitors: a scanning force microscopy
468 study, *Chemical Geology* 132 (1996) 227–236. URL: <https://www.sciencedirect.com/science/article/pii/S0009254196000599>. doi:10.1016/S0009-2541(96)00059-9.
- 469
- 470 [10] C. Hall, D. C. Cullen, Scanning force microscopy of gypsum dissolution and crystal growth, *AIChE Journal* 42 (1996)
471 232–238. doi:10.1002/aic.690420119.
- 472 [11] E. Finot, E. Lesniewska, J.-C. Mutin, J.-P. Goudonnet, Reactivity of gypsum faces according to the relative humidity by
473 scanning force microscopy, *Surface Science* 384 (1997) 201–217. URL: [http://www.sciencedirect.com/science/](http://www.sciencedirect.com/science/article/pii/S0039602897002203)
474 [article/pii/S0039602897002203](http://www.sciencedirect.com/science/article/pii/S0039602897002203). doi:10.1016/S0039-6028(97)00220-3.

- 475 [12] E. Finot, E. Lesniewska, J.-C. Mutin, J.-P. Goudonnet, Investigations of surface forces between gypsum crystals in electrolytic
476 solutions using microcantilevers, *The Journal of Chemical Physics* 111 (1999) 6590–6598. doi:10.1063/1.479950.
- 477 [13] E. Finot, E. Lesniewska, J.-P. Goudonnet, J. C. Mutin, Correlation between surface forces and surface reactivity in the setting
478 of plaster by atomic force microscopy, *Applied Surface Science* 161 (2000) 316–322. URL: <http://www.sciencedirect.com/science/article/pii/S0169433200000301>. doi:10.1016/S0169-4332(00)00030-1.
479
- 480 [14] E. Finot, E. Lesniewska, J.-C. Mutin, J.-P. Goudonnet, Investigations of surface forces between gypsum microcrystals in air
481 using atomic force microscopy, *Langmuir* 16 (2000) 4237–4244. doi:10.1021/1a9902439.
- 482 [15] E. A. Pachon-Rodriguez, A. Piednoir, J. Colombani, Pressure solution at the molecular scale, *Phys. Rev. Lett.* 107 (2011)
483 146102. doi:10.1103/PhysRevLett.107.146102.
- 484 [16] J. Adrien, S. Meille, S. Tadier, E. Maire, L. Sasaki, In-situ X-ray tomographic monitoring of gypsum plaster setting,
485 *Cement and Concrete Research* 82 (2016) 107–116. URL: <http://www.sciencedirect.com/science/article/pii/S0008884615003051>. doi:10.1016/j.cemconres.2015.12.011.
486
- 487 [17] J. Seiller, T. Bonnal, J. Adrien, S. Meille, S. Tadier, E. Maire, A. Bonnin, 4D in situ monitoring of the setting of α
488 plaster using synchrotron X-ray tomography with high spatial and temporal resolution, *Construction and Building Ma-*
489 *terials* 304 (2021) 124632. URL: <https://www.sciencedirect.com/science/article/pii/S0950061821023874>.
490 doi:10.1016/j.conbuildmat.2021.124632.
- 491 [18] M. La Bella, R. Besselink, J. P. Wright, A. E. S. Van Driessche, A. Fernandez-Martinez, C. Giacobbe, Hierarchical syn-
492 chrotron diffraction and imaging study of the calcium sulfate hemihydrate – gypsum transformation, *Journal of Applied*
493 *Crystallography* 56 (2023). doi:10.1107/S1600576723002881.
- 494 [19] J. Schindelin, I. Arganda-Carreras, E. Frise, V. Kaynig, M. Longair, T. Pietzsch, S. Preibisch, C. Rueden, S. Saalfeld,
495 B. Schmid, J.-Y. Tinevez, D. J. White, V. Hartenstein, K. Eliceiri, P. Tomancak, A. Cardona, Fiji: an open-source plat-
496 form for biological-image analysis, *Nature Methods* 9 (2012) 676–682. doi:10.1038/nmeth.2019.
- 497 [20] A. Fantou, A. Wozniak, A. Malchère, L. Roiban, S. Meille, K. Masenelli-Varlot, S. Tadier, Timelapse: Crystal growth
498 during setting of gypsum plaster, observed through in-cell environmental SEM (with setting retardant), 2021. URL: <https://hal.science/hal-04168810>.
499
- 500 [21] A. Fantou, A. Wozniak, A. Malchère, L. Roiban, S. Meille, K. Masenelli-Varlot, S. Tadier, Crystal growth during setting
501 of gypsum plaster, observed through in-cell environmental sem (without any setting retardant), 2021. URL: <https://hal.science/hal-04168814>.
502
- 503 [22] F. Otálora, J. García-Ruiz, Nucleation and growth of the Naica giant gypsum crystals, *Chem. Soc. Rev.* 43 (2014) 2013–2026.
504 doi:10.1039/C3CS60320B.
- 505 [23] B. Madeja, J. Avaro, A. E. S. Van Driessche, M. Rückel, E. Wagner, H. Cölfen, M. Kellermeier, Tuning the growth
506 morphology of gypsum crystals by polymers, *Cement and Concrete Research* 164 (2023) 107049. URL: <https://www.sciencedirect.com/science/article/pii/S0008884622003416>. doi:10.1016/j.cemconres.2022.107049.
507
- 508 [24] K. Momma, F. Izumi, *VESTA 3* for three-dimensional visualization of crystal, volumetric and morphology data, *Journal of*
509 *Applied Crystallography* 44 (2011) 1272–1276. doi:10.1107/S0021889811038970.
- 510 [25] P. F. Henry, M. T. Weller, C. C. Wilson, Neutron powder diffraction in materials with incoherent scattering: an illustration
511 of Rietveld refinement quality from nondeuterated gypsum, *Journal of Applied Crystallography* 42 (2009) 1176–1188.
512 doi:10.1107/S0021889809043210.
- 513 [26] X. Chen, J. Gao, Y. Zhao, Investigation on the hydration of hemihydrate phosphogypsum after post treatment, *Con-*
514 *struction and Building Materials* 229 (2019) 116864. URL: <http://www.sciencedirect.com/science/article/pii/S0950061819323001>. doi:10.1016/j.conbuildmat.2019.116864.
515
- 516 [27] C. Liu, J. Gao, X. Chen, Y. Zhao, Effect of polysaccharides on setting and rheological behavior of gypsum-based materials,
517 *Construction and Building Materials* 267 (2021) 120922. URL: <http://www.sciencedirect.com/science/article/pii/S0950061820329275>. doi:10.1016/j.conbuildmat.2020.120922.
518

- 519 [28] D. Alloyeau, W. Dachraoui, Y. Javed, H. Belkahla, G. Wang, H. Lecoq, S. Ammar, O. Ersen, A. Wisnet, F. Gazeau, C. Ri-
520 colleau, Unravelling kinetic and thermodynamic effects on the growth of gold nanoplates by liquid transmission electron
521 microscopy, *Nano Letters* 15 (2015) 2574–2581. doi:10.1021/acs.nanolett.5b00140.
- 522 [29] C. Vellmer, B. Middendorf, N. Singh, Hydration of α -hemihydrate in the presence of carboxylic acids, *Journal of Thermal*
523 *Analysis and Calorimetry* 86 (2006) 721–726. doi:10.1007/s10973-005-7224-4.
- 524 [30] K. M. Song, J. Mitchell, H. Jaffel, L. F. Gladden, Simultaneous monitoring of hydration kinetics, microstructural evolution,
525 and surface interactions in hydrating gypsum plaster in the presence of additives, *Journal of Materials Science* 45 (2010)
526 5282–5290. doi:10.1007/s10853-010-4572-7.
- 527 [31] M. Lanzón, P. A. García-Ruiz, Effect of citric acid on setting inhibition and mechanical properties of gypsum building
528 plasters, *Construction and Building Materials* 28 (2012) 506–511. URL: [http://www.sciencedirect.com/science/
529 article/pii/S0950061811003357](http://www.sciencedirect.com/science/article/pii/S0950061811003357). doi:10.1016/j.conbuildmat.2011.06.072.
- 530 [32] P. Abellan, T. J. Woehl, L. R. Parent, N. D. Browning, J. E. Evans, I. Arslan, Factors influencing quantitative liquid (scanning)
531 transmission electron microscopy, *Chem. Commun.* 50 (2014) 4873–4880. doi:10.1039/C3CC48479C.
- 532 [33] T. J. Woehl, T. Moser, J. E. Evans, F. M. Ross, Electron-beam-driven chemical processes during liquid phase transmission
533 electron microscopy, *MRS Bulletin* 45 (2020) 746–753. doi:10.1557/mrs.2020.227.

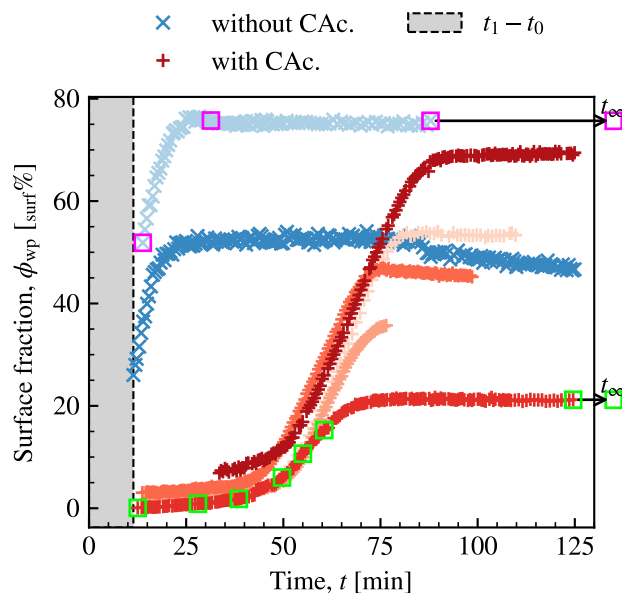
534 **6. Supplementary information**

Figure S1: Evolution of ϕ_{wp} , (non-normalized) surface fraction of white pixels for the different experiments, between t_1 and t_{last} . Blue crosses and red plus markers represent experiments conducted without ($N = 2$, pale and dark blue) and with ($N = 5$, different shades of red) citric acid (CAC), respectively. $\phi_{wp}(t_{\infty})$ was not computed as microographies at t_{∞} were recorded with different conditions, several days after t_{last} . For experiment details, refer to table 1. The magenta and green squares highlight times at which were collected the microographies displayed in figs. 2(b) and 3(b), respectively. The gray area represents the dead-time between the mixing time t_0 and the first acquisition t_1 . Reproduced from doi: 10.6084/m9.figshare.23723673 © (2023) A. Fantou, CC BY 4.0 license <https://creativecommons.org/licenses/by/4.0/>.

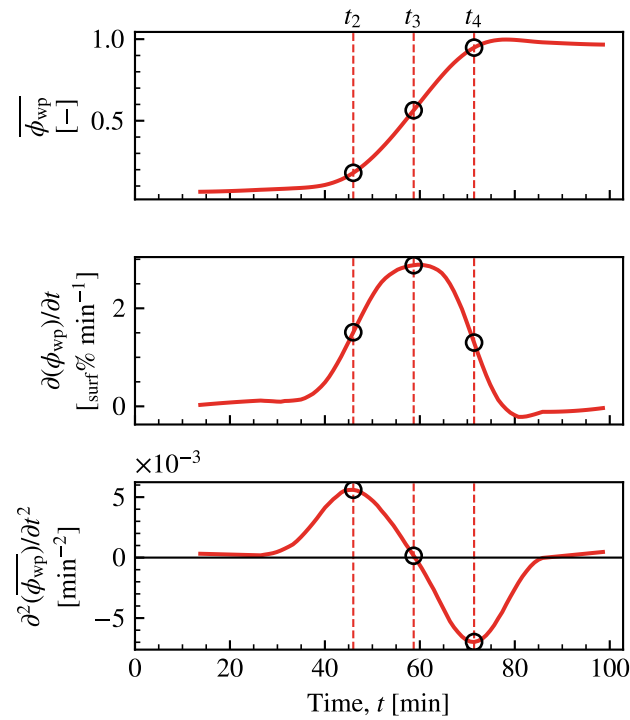


Figure S2: Illustration of the determination of the three characteristic times: t_2 (end of induction regime), t_3 (inflection point in the acceleration regime) and t_4 (start of termination regime: $\overline{\phi_{wp}}$ reaches a plateau). These times are chronologically identified when the second derivative of $\overline{\phi_{wp}}$ (surface fraction of white pixels normalized with respect to the maximum value of $\overline{\phi_{wp}}$), $\frac{d^2(\overline{\phi_{wp}})}{dt^2}$ is maximum, null and minimum, respectively. Computed values of t_2 , t_3 and t_4 are provided in table 2. Reproduced from doi: [10.6084/m9.figshare.23723673](https://doi.org/10.6084/m9.figshare.23723673) © (2023) A. Fantou, CC BY 4.0 license <https://creativecommons.org/licenses/by/4.0/>.

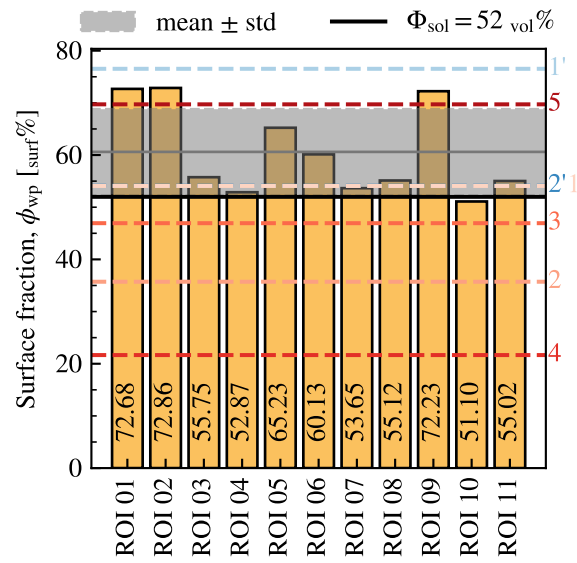


Figure S3: Surface fraction of solid (*i.e.*, crystals) of different ROI arising from 2D slices of a 3D volume obtained from μ -CT. Each ROI has the same dimension (130×70) μm^2 , which is equivalent to ($433 \text{ pix} \times 233 \text{ pix}$) for a voxel size of $0.3 \mu\text{m}$. The gray range highlights the mean value \pm the standard deviation of the surface fractions ($N = 11$). The dashed lines corresponds to the maximum surface fraction reached in the different experiments, which numbers are given on the right (see table 1). The black solid line indicates the theoretical volume fraction of solid Φ_{sol} computed from eq. (4). Reproduced from doi: [10.6084/m9.figshare.23723673](https://doi.org/10.6084/m9.figshare.23723673) © (2023) A. Fantou, CC BY 4.0 license <https://creativecommons.org/licenses/by/4.0/>.

535 6.1. Details on image processing

536 Several steps were needed to obtain hemihydrate binary masks. They are described hereafter.

537 6.1.1. First step: build a binary image for hemihydrate particles from the image acquired at time t_1

538 A simple threshold was applied on the image stack to separate crystals from the solution (see fig. S4
539 - R01, R41, A01, A41). At this step, the hemihydrate (HH) particles could not be distinguished from
540 dihydrate (DH) crystals. Indeed, their compositions are quite similar (they differ only by the amount of
541 water molecules), giving the crystals very similar gray levels. To segment the HH particles and not the DH
542 crystals, we considered the fact that HH dissolves during the reaction, whereas the DH germinates and grows
543 in size (see eq. (1)). Hence, by subtracting the binary image corresponding to time $t_{41} = 1$ h, 3 min, 6 s
544 (A41) to the first binary image (time $t_1 = 29$ min, 15 s) (A01), pixels corresponding to HH, which disappear
545 during this period of time (t_1 to t_{41}), became black, see A01. The whole HH crystals at time t_1 could then
546 be identified, as they were bound by the contours of image A01 (contours shown in image B01) and contain
547 non-white pixels. Image E was created as the average from images B01 and C. A quick manual filling
548 in black of these HH regions was then carried out. A separation between HH particles and DH crystals
549 (white pixels zones with gray contours in E) was then made by thresholding. The binary image showing
550 HH particles at time t_1 is shown in M. Image F01 shows the overlay of the HH mask with red contours on
551 the SEM image R01.

552 6.1.2. Second step: build binary masks for hemihydrate particles for each image

553 This step had to be applied to follow the evolution of HH particles and Dh crystals in function of the
554 reaction time, see fig. S5.

555 The logical AND operation was applied to the binary mask of HH obtained from the image at time t_1
556 (image M) and each binary image of the first stack (segmented stack, containing all crystals in black on
557 a white background representing the solution) (see fig. S5, images A05 - A20 - A33). The result of this
558 operation correctly matches the evolution of HH particles over time in different images (see F05 and F20),
559 until DH crystals grow and cover the HH areas (see yellow arrows in image F33). In order to fix this issue,
560 we thresholded big needles of gypsum at large times (*e.g.*, $t > t_{30}$). These binary images of DH at times
561 could then be subtracted to the binary images of non-perfect HH. At the end, a binary stack of good quality
562 could be obtained to monitor the evolution of HH particles on the one side (see F'33), and DH crystals on
563 the other side.

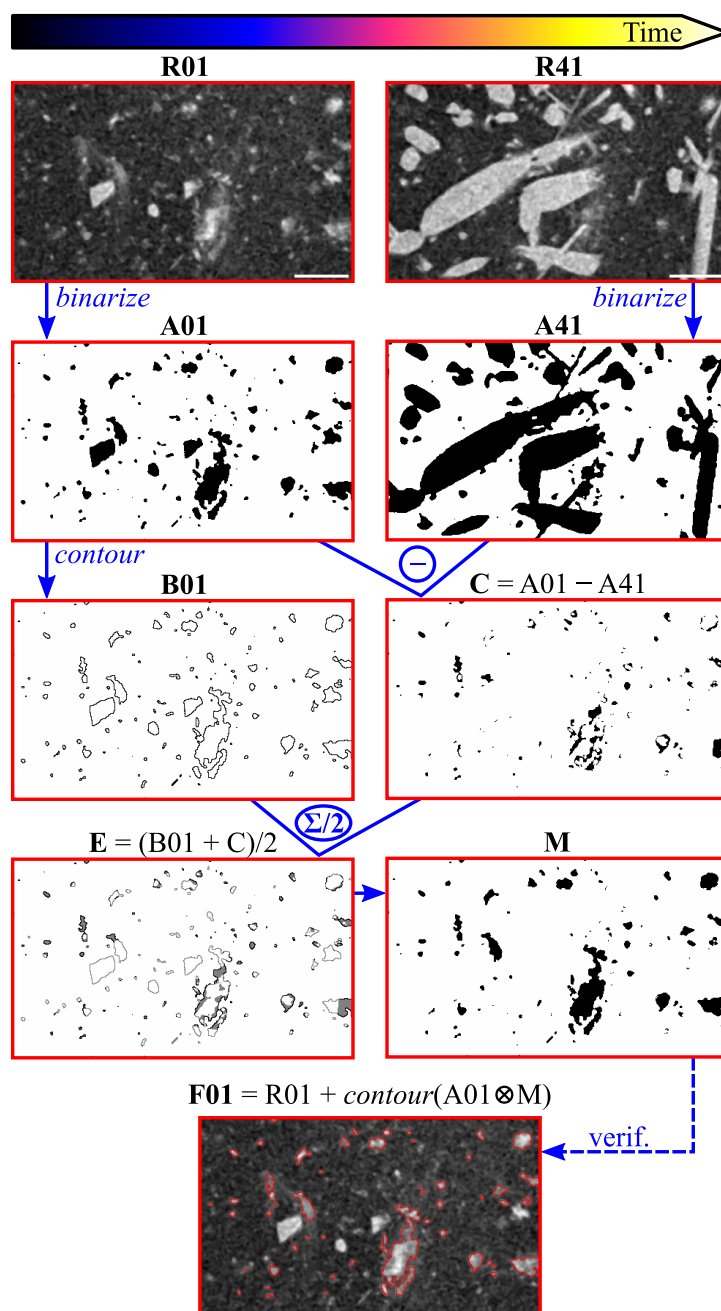


Figure S4: Illustration of the different steps to obtain a binary mask (denoted M) of HH particles from the raw image (denoted R) acquired at time t_1 (R01). R01 = 1st raw image of the experiment 1, collected at time t_1 (scale bar 10 μm). R41 = 41st raw image of the same region of interest (scale bar 10 μm). A01 = binary image obtained from R01 (time t_1). A41 = binary image obtained from R41. B01 = contours of the black particles in A01. C = A01 - A41. E = average of images B01 and C. M = HH binary mask at time t_1 . F01 = overlay of the mask M on the raw image R01 to verify the quality of the mask M. Reproduced from doi: 10.6084/m9.figshare.23723673 © (2023) A. Fantou, CC BY 4.0 license <https://creativecommons.org/licenses/by/4.0/>.

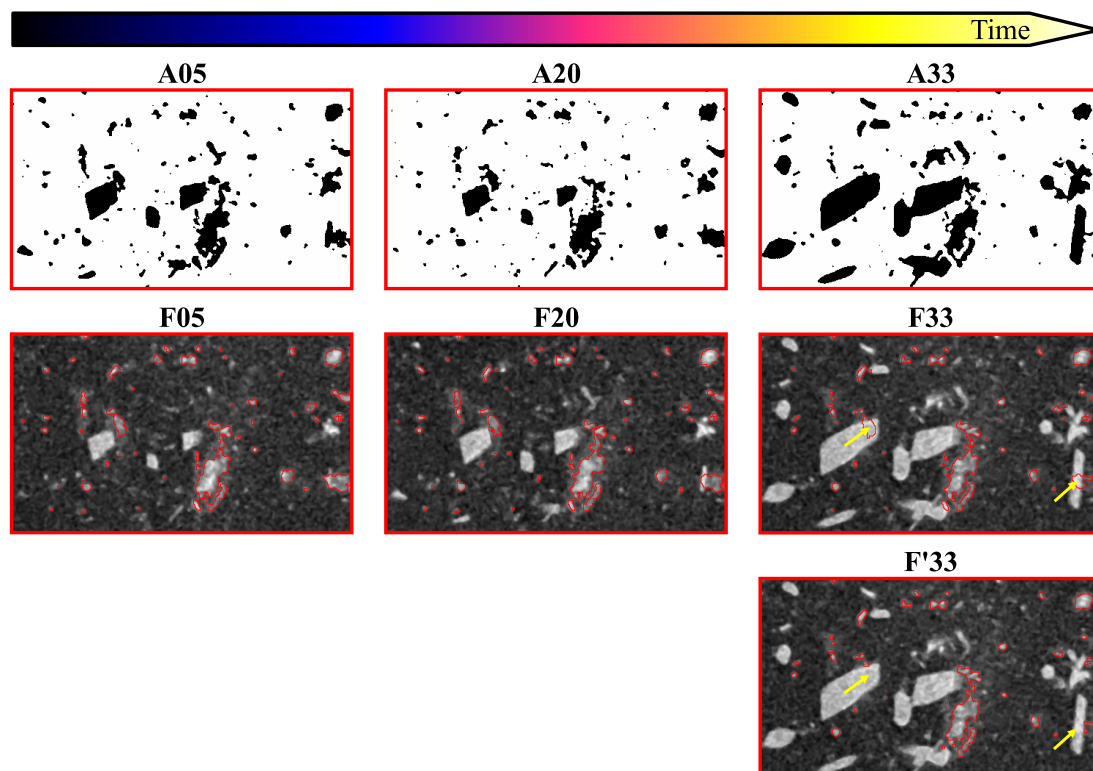


Figure S5: Illustration of the different steps to obtain a stack of binary images of HH particles. A05 = 5th binary image with all crystals in black. F05 = result (in red) of the AND operation between images M (see fig. S4 for a reminder on how were obtained the F images) and A5, overlaid to the 5th raw image (corresponding to R05). A20 = 20th binary image with all crystals in black. F20 = result (in red) of the AND operation between images M and A20, overlaid to the 20th raw image (corresponding to R20). A33 = 33rd binary image with all crystals in black. F33 = result (in red) of the AND operation between images M and A33, overlaid to the 33rd raw image (corresponding to R33). Yellow arrows show regions where DH crystals and HH mask overlap. F'33 = final mask of the HH particles obtained after subtraction from F33 of the regions where DH crystals and HH mask overlapped. No overlap is visible anymore, see yellow arrows. Reproduced from doi: [10.6084/m9.figshare.23723673](https://doi.org/10.6084/m9.figshare.23723673) © (2023) A. Fantou, CC BY 4.0 license <https://creativecommons.org/licenses/by/4.0/>.

564 6.2. Evaluation of the detection depth using Monte Carlo simulations

565 Monte Carlo simulations using Hurricane software (from SAMx, Trappes, France) were performed to
 566 estimate below which depth DH crystals cannot be seen anymore in the Backscattered Electron images.
 567 To model the sample in the Qantomix cell, as no information was disclosed by the manufacturer, we
 568 assumed that the top membrane was 100 nm thick and made of a carbon-based polymer. The size of the
 569 computation box was set to 20 μm along each direction. The electron beam energy was set to 10 keV, as in
 570 the experiments, and 1 000 000 electron trajectories were computed. All electrons escaping the upper side
 571 of the computation box were considered as backscattered electrons. Different sample configurations were
 572 studied, with a dihydrate crystal immersed in water at different depths below the membrane (see table S1).

Table S1: Results obtained from Monte Carlo simulations performed on different computation boxes containing a dihydrate crystal immersed in water in a Qantomix cell. The thicknesses of water (W) and dihydrate (DH) are specified in each case within brackets. F_i , F_w and F_d are the fractions of backscattered electrons for the configuration i , pure water (configuration #1) and pure dihydrate (configuration #2), respectively.

Configuration number, i	Composition of the computation box					
	1	2	3	4	5	6
Water (W)	20 μm	–	–	200 nm	400 nm	900 nm
Dihydrate (DH)	–	20 μm	500 nm	500 nm	500 nm	500 nm
Water (W)	–	–	19.5 μm	19.3 μm	19.1 μm	18.6 μm
Fraction of BSE, F [%]	9.28	15.31	15.35	13.04	10.86	9.26
Visibility of DH crystal, N [%]	0	100	100	62	26	0
Gray level	0	255	255	158	66	0

$$N = (F_i - F_w)/(F_d - F_w)$$

573 The fractions of backscattered electrons obtained from the simulations for each sample configuration
 574 are reported in the table S1 by the F parameter. When the cell is filled with water only (configuration #1,
 575 in table S1), 9.28% of the incident electrons are backscattered. This rate can be associated with regions in
 576 black (gray level set to 0) in the acquired images, as we wanted to maximize the contrast between water and
 577 crystals. When the cell is filled only with DH (configuration #2, in table S1), or contains a 500 nm thick layer
 578 of DH directly below the membrane (configuration #3, in table S1), the fraction of backscattered electrons
 579 reaches 15.3%. As this value is obtained for both configuration, we can conclude that the backscattered
 580 electrons come from the first 500 nm of dihydrate. We consider that it is the best detection of gypsum, and
 581 for both cases a gray level of 255 (*i.e.*, white) can be associated in the images.

582 In the case where the DH crystal is located far below the membrane, the fraction of backscattered elec-
 583 trons drops rapidly. It reaches the value found for water when the gypsum is 900 nm below the membrane.
 584 This means that gypsum is visible when it is located at 200 nm or 400 nm below the membrane, but it cannot
 585 be detected anymore at 900 nm. However, if we consider the gray level to be proportionnal to the visibility
 586 of DH crystal (N), the pixels with gypsum at a depth of 400 nm would have a gray level of 61. This is quite
 587 dark, and these pixels may not be considered as crystals. It seems more reasonable to consider that the DH
 588 crystals that are visible in the images come from a maximum depth of about 200 nm below the membrane,
 589 that is to say 300 nm from the top of the cell.