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**Datasheet for the decision of 9 July 2024**

**Case Number:** T 0601/22 - 3.4.03

**Application Number:** 19164718.9

**Publication Number:** 3522199

**IPC:** H01J37/20

**Language of the proceedings:** EN

**Title of invention:**
ELECTRON MICROSCOPY SAMPLE SUPPORT COMPRISING POROUS METAL FOIL

**Applicant:**
United Kingdom Research and Innovation

**Relevant legal provisions:**
EPC Art. 52(1), 56

**Keyword:**
Inventive step - (no) - effect not made credible within the whole scope of claim

**Decisions cited:**
G 0002/21, T 0116/18

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**Case Number: T 0601/22 - 3.4.03**

**D E C I S I O N**
**of Technical Board of Appeal 3.4.03**
**of 9 July 2024**

**Appellant:** United Kingdom Research and Innovation
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North Star Avenue
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**Representative:** Mewburn Ellis LLP
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**Decision under appeal:** **Decision of the Examining Division of the European Patent Office posted on 6 October 2021 refusing European patent application No. 19164718.9 pursuant to Article 97(2) EPC.**

**Composition of the Board:**

**Chairman** T. Häusser
**Members:** M. Ley
E. Mille

## Summary of Facts and Submissions

I. The appeal is against the decision of the examining division refusing European patent application No. 19 164 718 pursuant to Article 97(2) EPC.

II. The examining division cited *inter alia* the following documents:

D1 E. Ermantraut et al., "Perforated support foils with pre-defined hole size, shape and arrangement", Ultramicroscopy, vol. 74, no. 1-2, 1 July 1998, pages 75 to 81, ISSN: 0304-3991, DOI: 10.1016/S0304-3991(98)00025-4

D2 S. Janbroers et al., "Preparation of carbon-free TEM microgrids by metal sputtering", Ultramicroscopy, vol. 109, no. 9, 1 August 2009, pages 1105 to 1109, ISSN: 0304-3991

D3 US 2011/200787 A1

D7 K. Naydenova et al., "Multifunctional graphene supports for electron cryomicroscopy", Proc. of the National Academy of Sciences, 24 May 2019, ISSN: 0027-8424, DOI: 10.1073/pnas.1904766116

D9 K. Naydenova et al., "Electron cryomicroscopy with sub-Ångström specimen movement", Science, 9 October 2020, pages 223 to 226, DOI: 10.1126/science.abb7927

The examining division decided that the subject-matter of claim 1 of the main request and of the first to third auxiliary requests lacked an inventive step (Article 56).

III. The appellant requests that the impugned decision be set aside and a European patent be granted on the basis of the main request or the first to third auxiliary requests filed with the statement setting out the grounds of appeal and corresponding to the requests underlying the impugned decision.

With the statement setting out the grounds of appeal, the appellant filed the following documents:

D10 Declaration of Ch. Russo dated 14 February 2022
D11 Pages of laboratory notebook of Ch. Russo dated 14 March 2012, 26 October 2012 and 8 November 2012

IV. Claim 1 according to the main request has the following wording (board's feature labelling):

*An electron microscopy sample support comprising:*
**(a)** *a support member comprising a plurality of spaced support elements arranged to form a mesh; and*
**(b)** *a metal foil comprising a porous region;*
**(c)** *wherein the support member, support elements and metal foil all being formed from the same metal and*
**(d)** *said support member being configured to give structural stability to said metal foil,*
**(e)** *said porous region of said metal foil being configured to receive an electron microscopy sample and*
**(f)** *wherein said support further comprises a graphene layer in ohmic contact with said metal foil.*

Claim 1 according to the first auxiliary request is identical to claim 1 according to the main request except that it specifies that the electron microscopy sample support is a cryo-electron microscopy sample support.

Claim 1 according to the second auxiliary request is identical to claim 1 according to the first auxiliary request except that feature (c) is amended as follows (additions underlined by the board):

**(c')** *wherein the support member, support elements and metal foil all being formed from the same metal comprising at least one of gold, platinum, palladium, rhodium or hafnium metal and*

Claim 1 according to the third auxiliary request is identical to claim 1 according to the first auxiliary request except that feature (c) is amended as follows (deletions/additions highlighted by the board):

**(c'')** *wherein the support member, support elements and metal foil all being formed from ~~the same metal~~gold and*

V. The appellant argument's can be summarized as follows:

Distinguishing features (c)/(c')/(c'') and (f) provided a synergistic effect, which was confirmed by post-published documents D7 and D9, published in 2019 and 2021, respectively.

The skilled person, having the common general knowledge in mind, and based on the application as originally filed, would derive said effect as being encompassed by the technical teaching and embodied by the same originally disclosed invention so that that the requirements of the order of the decision G 2/21 of the Enlarged Board of Appeal were met.

Starting from D1, it was not obvious for the skilled person to achieve this effect by the claimed subject-matter. An inventive step (Article 56 EPC) should thus be acknowledged.

## Reasons for the Decision

1. The invention concerns a electron microscopy (EM) sample holder, in particular for cryo-electron microscopy.

Cryo-electron microscopy can be used to produce 3D reconstruction images of samples suspended in vitreous ice, particularly biological specimens.

In preparation for cryo-EM, a sample is suspended in water which forms a thin layer across the small holes in an EM grid and is supported by surface tension of the water. In order to fix the sample in the grid hole for imaging, the grid is cooled rapidly to freeze the water, typically by plunging it into liquid nitrogen or ethane. The plunging is performed quickly, resulting in incredibly fast freezing to give "vitreous ice" in which the water molecules have not had time to reorganise into a crystalline form during the freezing process and undergo the typical volume expansion observed during slower freezing.

The invention aims at addressing some issues in relation with conventional porous carbon specimen supports (electron beam-induced motion of individual particles, charge accumulation on the specimen induced by the electron beam, chemical transformation of a specimen support), see e.g. Figures 1 to 3 and page 10, line 35 to page 11, line 25 of the description of the application.

As claimed the electron microscopy sample support comprises a support member with a plurality of spaced support elements arranged to form a mesh (see e.g.

Figures 4b and 5a of the application) and a metal foil comprising a porous region (see e.g. Figures 4b and 5b of the application). The support member, support elements and metal foil are all formed from the same metal, e.g. gold. The porous region of the metal foil receives the electron microscopy sample (e.g. a vitreous ice layer with biological material to be imaged), while the support member provides mechanical support.

As claimed, the support further comprises a graphene layer in ohmic contact with said metal foil.

2. The examining division and the appellant started from document D1 in their assessment of inventive step of claim 1 according to the main request. The board has no reasons to deviate from this choice.

3. It appears undisputed that D1 discloses all features of claim 1 of the main request except features (c) and (f).

Indeed, using the wording of claim 1, document D1 discloses an electron microscopy sample support (abstract, "Perforated support foils with holes of pre-defined size, shape and arrangement and with hole sizes down to the sub-micrometer range, named Quantifoil, are presented", "The foils are particularly beneficial in electron microscopy, when a specimen support is required with holes smaller than those attainable with metal grids", "Their advantages in cryo-electron microscopy, for example, are illustrated"; sections "2. The fabrication of Quantifoil" and "4. Results"; Figure 2) comprising:

a support member comprising a plurality of spaced

support elements arranged to form a mesh (page 76, right-hand column, last paragraph, "EM grids"; Figure 2B and description thereof, "grid bar"); and
a metal foil comprising a porous region (page 76, right-hand column, last paragraph, "The photoresist layer is picked up onto EM grids, dried, and coated with 15—20 nm carbon or other materials, e.g. gold, by vacuum deposition techniques"; page 77, section "4. Results", first paragraph, "The scanning EM image in Fig. 2B shows a gold foil with circular holes with a diameter of 2.1 µm, separated by 1.9 µm wide bars"; Figure 2B and description thereof, "gold foil");

said support member being configured to give structural stability to said metal foil (implicit),

said porous region of said metal foil being configured to receive an electron microscopy sample (page 77, left-hand column, last paragraph; page 78, left-hand column, "Quantifoil was tested preparing vitrified specimens of biological suspensions; free-hanging, vitreous ice layers in the holes of the foil were prepared according to the so-called perforated foil technique").

As D1 only discloses that a gold foil is used, but does not disclose which material is used for the EM grid, the sample holder of D1 lacks feature (c), i.e. that the support member, support elements and metal foil all being formed from the same metal. A graphene layer as specified in feature (f) is not mentioned, either.

4. There is a disagreement between the examining division and the appellant whether an inventive step should be acknowledged on the basis of these two distinguishing features.

5. The examining division argued that the post-published document D7 allegedly disclosing a synergistic effect was to be disregarded. They referred to section I.D. 4.4.2 of the Case Law of the Boards of Appeal, 9th Edition, 2019. They argued that, although "the advantage obtained by the combination of graphene and an all-gold support" did not alter the character of the invention, the effect could not be unambiguously deduced by the skilled person from the original application in the light of the closest prior art and it was not at least hinted at in that application. The criteria of said section of the Case Law of the Boards of Appeal were not met in the present case.

The examining division held that each of features (c) and (f) was obvious so that, as a consequence, the subject-matter according to the main request and the auxiliary requests lacked an inventive step (Article 56 EPC).

6. The appellant argued that an inventive step should be acknowledged on the basis of a synergistic effect when a sample support member, support elements and metal foil were formed from the same metal and a graphene layer in ohmic contact with the metal foil was added to the sample support, i.e. when the sample support was constructed in accordance with features (c) and (f).

6.1 The appellant argued in particular that the problem addressed by features (c) and (f) was to reduce particle motion due to a build-up stress in the vitreous ice layer released during electron beam irradiation, i.e. during imaging.

The application was directed to reducing particle motion effects during cryo-EM imaging, whereby an increased resolution was achieved, see page 1, lines 33 to 36; page 17, lines 31 to 36; Figures 7, 9a and 9d of the application documents. Figures 9a and 9d showed a reduction in motion of a biological sample when an EM grid having the construction according to feature (d) (gold in this case) was used (Figure 9d) as compared to the greater motion when a standard amorphous carbon EM grid was used (Figure 9a). By reducing the particle motion during application of an electron beam, an increased resolution per particle could be achieved allowing more accurate 3D reconstructions of a sample to be produced.

The motion of particles during imaging was associated with built-up stresses in an ice layer used in cryo-EM applications which were released during imaging on application of an electron beam. These effects were minimised by using the claimed sample support and were not mentioned in D1.

During the rapid cooling used when preparing a sample for cryo-EM, buckling could occur in two different elements of the system: the film supporting the ice and the ice layer itself.

Considering the film supporting the ice layer, e.g. the metal foil, cryo-crinkling or puckering could occur when the thermal expansion coefficient of the grid bars or structures supporting the film differed from that of the film itself. This resulted in differential movement of the support and the film resulting in build-up of stresses in the film and, eventually, puckering. This was the problem discussed in D1 in the section bridging pages 80 and 81 and led to a grid which was not flat before introduction into a microscope. It did not relate to the induction of motion of particles during imaging when an electron beam was applied.

Considering the ice layer itself, buckling of the ice sheet could occur separately from motion of the supporting film and was due to different thermal expansion coefficients of the ice and the surrounding support film (which defined the hole in which the ice was suspended). Contraction of the surrounding film placed stress on the ice layer resulting, eventually, in its buckling. The appellant referred to Figure 2 of post-published document D9 and argued that this effect was not mentioned in D1 and that it was important in the consideration of sample motion during imaging. These additional stresses (formed in the ice sheet when it was further cooled below the homogeneous nucleation point, see Figure 2 of post-published document D9) could be released upon electron beam application during imaging and resulted in movement of the sample. The irreversible release of stresses was thus induced by the electron beam (at an effective temperature of 147 K as shown in Figure 2 of D9).

6.2 The appellant argued that the technical problem due to undesired stress release was unexpectedly solved in a synergistic way by features (c) and (f), as shown in the post-published document D7.

By forming a support including the foil from the same metal such that a uniform thermal expansion coefficient (TEC) was achieved, surprisingly the additional stresses in the ice sheet that could be released upon electron beam application during imaging were beneficially reduced. This beneficial effect of reduction in the released stresses of an ice sheet during imaging and the consequential reduction in particle motion was not described in D1, and implied an increase in image resolution.

According to the appellant, surprisingly, there was a further synergistic improvement in the reduction of the motion of particles when a graphene layer was used in combination with the sample support being formed from the same metal. This synergistic effect was evidenced by the post-published data of D7, see Figure S14 submitted during examination, panels A to E. Panels B and C showed the effect of providing an graphene layer on a conventional carbon support and panels B and D showed the effect of using a support made of gold. According to D7, page 11720, right column, adding a graphene layer to an all-gold support reduced particle movement by a factor of 2 during electron irradiation (see Figure S14, panels D and E). Compared with graphene-on-carbon supports, graphene-on-gold supports reduced particle movement by a factor of 3 (see Figure S14, panels C and E).

By adding a graphene layer to a sample support which had a vastly different TEC with respect to graphene, the uniform TEC of the sample support would be disrupted. A skilled person would not expect a beneficial reduction of motion of a sample. The limited improvement in stability due to the increase in mechanical stability provided by a graphene layer would not be expected to offset the increase in motion of particles caused by disrupting the uniform TEC. The skilled person would not expect even the maintenance of the effect seen for a stable same metal grid when adding a graphene layer, let alone the significant further reduction in sample movement effect seen, as evidenced by D7.

6.3 D7 was to be taken into account, as the technical effect provided by features (c) and (f) was hinted at or foreshadowed in the application and did not change the nature of the invention.

6.3.1 As pointed out by the appellant, the examining division accepted that D7 provided evidence for a synergistic technical effect achieved by features (c) and (f).

According to the appellant, the examining division had erred in their reasoning when they asserted that the data of D7 could not be taken into account.

The examining division erroneously argued that the case law could not be straightforwardly applied to the technical field of the invention and the effect was not hinted at in the application as filed. There were no indications or suggestions in the case law of the Boards of Appeal or the EPO Guidelines that the guidance provided in relation to post-filed data was only applicable to the technical field of chemistry, as argued by the examining division.

6.3.2 Moreover, the synergistic effect provided by features (c) and (f) met the requirements for consideration of a "new" effect in the EPO Guidelines [2021] G-VII, 11 for the reason that the synergistic effect related to the technical problem initially suggested in the application as filed. A technical problem which the application aimed to solve was the provision of improved sample stability during cryo-EM imaging. The data of D7 was clearly related to this technical problem. It presented clear evidence of further stabilisation of a sample during cryo-EM imaging. Reference was also made to several decision of the

Boards of Appeal and the Guidelines [2021] G-VII, 5.2, fifth paragraph.

6.3.3 Moreover, the advantage related to the same field of use of the application as filed, the field of use of the effect/advantage and the application being cryo-EM supports, and did not alter the character of the invention because the technical problem specified in the application was supplemented by a further improvement of sample stability demonstrated by the combination of features (c) and (f).

6.3.4 The effect was thus also hinted at in the application and the problem was "foreshadowed in the application", contrary to the examining division's position. No further hint was necessary. Hence, all criteria given in the Case Law of the Boards of Appeal, I.D.4.4.2, 9th edition, 2019 were met. Several decisions were cited. It was not necessary that the synergistic effect could be unambiguously deduced from the original application.

6.3.5 It was irrelevant that the authors of D7 only posited about the underlying forces leading to the synergistic reduction in motion for the graphene on gold specimen support when an electron beam was applied (see page 11721, left hand column of D7). D10 and D11 further confirmed that the synergistic effect of features (c) and (f) was known to the inventors at the priority date. The page in the laboratory notebook D11 dated 14 March 2012 showed a first attempt to make graphene on a porous metal foil which was then to be attached to a grid, the page dated 26 October 2012 showed the complete description of this support and the page dated 8 November showed a first experimental micrograph, which was an image of 70S ribosomes frozen in amorphous water. Hence, at the priority date, the inventors were "in possession of the invention".

6.3.6 Moreover, the skilled person would also conclude that the application suggested the advantageous embodiments where features (c) and (f) were combined together due to the wording and positioning of the paragraphs of the application, see page 5, lines 33 to page 6, line 9; page 8, lines 29 to 34 and page 6, lines 11 to 29; page 8, line 35 to page 9, line 12. Reference was also made to page 6, lines 16 to 18 of the application, from which the skilled person would at least derive that providing a graphene layer would not increase the particle motion (and hence decrease the image quality).

The application and the examples were aimed at obtaining improved images during cryo-EM, i.e. improving the resolution per particle so that high resolution 3D reconstruction images could be achieved, see page 2, lines 8 to 11; page 17, lines 34 to 36; Figure 7. This was linked to preventing or reducing the motion of said particle during imaging. Hence, page 6, lines 16 to 18 foreshadowed the fact that a graphene layer did not decrease the image quality, while the skilled person would expect a decrease due to the uniform thermal expansion coefficient of the grid being disrupted by the graphene.

6.4 The appellant further argued that the conditions of the order of the decision G 2/21, when interpreted with the help of T 116/18, were met.

6.4.1 G 2/21 provided a thorough analysis of when post-published data was effective to demonstrate a technical effect. The assessment could be conceptually broken down into two stages. First, it had to be ascertained whether the post-published data provided evidence of a technical effect that could be derived from the application as filed, i.e. whether the skilled person, based on the application as filed, could derive that technical effect as being encompassed by the technical teaching and embodied by the same originally disclosed invention; and second, it had to be examined whether the post-published data (in D7, D10, D11) credibly demonstrated that technical effect.

6.4.2 The main guiding principle established in point 2 of the order of G 2/21 was that the skilled person, having regard to the common general knowledge, and based on the application as originally filed, would derive the relevant technical effect as being i) encompassed by the technical teaching and ii) embodied by the same originally disclosed invention, see also the Guidelines G-VII, 5.2 in the version of March 2024. Guidance about the implementation of point 2 was found in T 0116/18, Reasons 11.10, 11.11, 11.13.1.

6.4.3 In the present case, the technical effect supported by the data, i.e. the improved stability, was conceptually comprised by the broadest technical teaching of the application as filed, see page 1, lines 16 to 25 and lines 28 to 36 indicating that the application aimed to address some of the problems associated with known sample supports, including electron beam induced physical, chemical and/or electrical changes to the support and/or specimen which might have an impact on the resolution of images obtained by electron microscopy, e.g. the motion of the specimen, see also page 10, lines 28 to 32. Page 12, lines 6 to 9 mentioned an ultra-stable sample support which might address the problems, including the reduction of particle motion resulting from a difference in thermal expansion coefficient between the ice layer and the support by providing additional stability.

6.4.4 Moreover, the skilled person would have no legitimate reason to doubt that the purported technical effect could be achieved by the claimed subject-matter. There was no requirement for a direct and unambiguous disclosure of the technical effect in the application.

The appellant pointed out that the type of graphene or its functionalization were not important for achieving the technical effect. The functionalization of the graphene related to the surface compatibility with a given sample, not to the structural stability provided, which was retained independently of any surface modification or functionalization.

The improvement would also be obtained for metals other than gold.

6.4.5 Thus, when the facts and evidence of the present case were assessed with the criteria of G 2/21 in mind and informed by the application of those criteria in T 0116/18, it was clear that a skilled person would derive the presently alleged technical effect as being encompassed by the technical teaching and that it was embodied by the same originally disclosed invention.

Furthermore, the skilled person recognised that the technical effect was credibly demonstrated by the post-published evidence on file, such as D7, D10 and D11.

6.5 Hence, for the appellant, it was justified to reformulate the objective technical problem based on the synergistic effect provided by features (c) and (f).

Features (c) and (f) solved the objective technical problem of the provision of a sample support providing further improved sample stability during cryo-EM imaging. This solution was not obvious in view of D1 and D3.

D1 did not teach towards reducing the motion of particles during imaging at all, let alone how to further reduce the motion of particles during imaging. D1 instead related to preventing the puckering of a grid before imaging. D3 did not discuss the motion of particles during cryo-EM imaging or how this could be reduced, either.

7. The appellant also submitted that the claims were inventive based on the feature (c) alone.

D1 did not relate to the reduction of stresses in the ice layer. The cryo-crinkling or puckering described in paragraph 1 on page 81 of D1 was a different effect associated with the build-up and release of stresses in a film/foil during cooling of a grid. These stresses were dissipated by the puckering of a grid before imaging occurs and were not associated with the motion of particles during cryo-EM imaging itself. D1 did not disclose the important technical effect of reducing sample motion associated with feature (c).

The objective technical problem facing the skilled person was "the provision of improved sample stability during cryo-EM imaging".

It would not be obvious to solve this problem when starting from D1. This document did not teach formation of a support using a single metal to provide a uniform thermal expansion coefficient. An arrangement was described which was remote from the other teaching of the document and where a grid/foil was formed completely from a polymeric material.

The appellant alleged that, at the priority date of the application, the common understanding in the field was that when using an EM grid in practice, a grid comprising an amorphous material (e.g. an amorphous carbon foil) was required because the amorphous material on which to focus the electron beam was used as a standard at the start of the imaging. The skilled person would not think of using the foil fabricated in D1 to form a "same metal" cryo-EM grid with any expectation of forming a usable sample support because they would not expect to be able to focus the electron beam in the same standard way. The common understanding in the field at the time was that such a grid would present significant and unusual practical difficulties in focusing of the beam. It was only after investigation by the inventors of the current application that the incorrect preconception that an amorphous material had to be used in a sample support for focusing a beam during imaging was overturned.

Even if D1 taught to form a uniform TEC grid to reduce the puckering in a grid, that puckering was a different effect caused by different stresses to those which cause the motion of a sample during cryo-EM imaging. Due to the complexity of the cryo-EM system the skilled person would not think that just because forming a grid with a uniform TEC reduces the puckering of a grid before cooling that a grid with uniform TEC would also have an effect on the stresses applied and released from an ice sheet during imaging or that the motion of particles during imaging could be reduced.

None of the other pieces of prior art cited taught towards reducing the motion of particles during imaging or that this could be achieved by forming a sample support having feature (c).

The argumentation for the gold foil of D1 was equally valid for other metals.

8. The board is not convinced by the appellant's arguments and takes the view that the subject-matter of claim 1 according to the main request does not involve an inventive step.

8.1 In Figure S14 of D7, panel A represents the displacement of ribosomes (in Å) as a function of the electron fluence (in electrons per Å$^2$) for an amorphous carbon foil on an amorphous carbon support, panel B represents said displacement for suspended vitreous ice in a holey carbon foil on a copper support, panel C for graphene on a holey carbon foil on a copper support, panel D for a holey gold foil on a gold support (in accordance with feature (c)). These results corresponds to Figures 9a to 9d of the application. Panel E shows the displacement for a graphene sheet on a holey gold foil on a gold support and panel F represents the theoretical limit. Panel E corresponds to a support encompassed by claim 1 of the main request.

A comparison between panels (B) and (D), which correspond to Figures 9b and 9d of the application, indicates that the particle movement is reduced, because the carbon support is replaced by a gold support with the the thermal expansion coefficients of the metal foil and the grid being matched. This is the improvement discussed in the present application and results from both features (b) and (c). It should be noted that panel D in Figure S14 does not allow to conclude what would be the effect of feature (c) alone, because a comparative measurement e.g. for a gold foil on a copper support was not performed.

A comparison between panels (B) and (C), which correspond to Figures 9b and 9c of the application, shows the effect of adding a graphene layer on a conventional carbon support. Mechanical strengthening (as disclosed in the application, page 6, lines 16 to 19; page 15, lines 6 to 8) reduces the displacement.

Hence, panels (B) to (D) of Figure S14 of D7 describe the reduction in particle movement as discussed in Figures 9b to 9d of the application, i.e. the respective effects of features (b)/(c) and (f).

It is questionable whether an synergistic effect is shown in or deducible from Figure S14 of D7.

Two features interact synergistically if their functions are interrelated and lead to an additional effect that goes beyond the sum of the effects of each feature taken in isolation.

It is doubtful whether panel E of Figure S14 allows to conclude that a synergistic effect is present. For example, the addition of a graphene layer to the all-gold support (see panels D and E) reduces the RMS displacement by about 1 Å for a fluence of 15 electrons per Å$^2$. A similar reduction (of about 1 Å) is found when adding a graphene layer on a holey carbon foil (see panels B and C). It is thus arguable whether Figure S14 shows an additional effect that goes beyond the sum of the effects of each feature (c) and (f) taken in isolation.

In the following the board assumes, for the sake of the argument, that the alleged synergistic effect is present for the specific device shown in D7.

8.2 Furthermore, the board is sceptical whether the purpoted technical effect is disclosed in or can be deduced from the application as originally filed.

8.2.1 Page 6, lines 11 to 29 of the description of the application discusses an embodiment with a graphene layer. The passage in lines 16 to 18 states that possibly the graphene layer allows "for additional structural stability, whilst not degrading the quality of the resultant images", see also original claim 5. The board preliminarily cannot derive any (beneficial or detrimental) effect on the image quality, only an increase in mechanical stability might be possible ("may allow"). Page 6, lines 20 to 22 discloses that the impact of the graphene layer is minimised so that the "benefits of the porous metal foil" are maintained. No further effects were mentioned on page 8, line 35 to page 9, line 8; page 21, lines 4 to 17 of the application or in original claims 1 to 4, 6 to 19.

"Graphene devices" are also described from page 14, line 32 to page 15, line 29 of the description of the application. Page 14, lines 33 to page 15, line 8 states that providing a graphene layer in the support may decrease surface charge build up due to the conductive properties of the graphene or may increase "mechanical strength of the substrate", see also page 15, lines 23 to 25.

Page 18, line 31 of the application states that Figure 9c relates to a sample supported on a graphene substrate. No graphene layer on a porous metal foil is mentioned. Hence, none of the figures of the application show experimental evidence of the alleged technical effect.

In other words, no effect of a graphene layer on the particle motion or the image quality is mentioned in or derivable from the content of the application. The only effect credibly achieved is the provision of "additional structural stability".

8.2.2 According to the appellant, the particle motion effects during cryo-EM imaging described in D7 were associated with built-up stresses in an ice layer, said stresses were due to different thermal expansion coefficients of the ice and the surrounding support film. The application was allegedly directed to the same effect.

The board however notes that on page 1, lines 10 to 22 of the application it is stated that the invention concerns the reduction of undesired physical, chemical and/or electrical changes to the support and/or specimen by the electron beam; such changes may impact results, including resolution of image, obtained through use of electron microscopy techniques. Page 1, line 33 to page 2, line 2 mentions electron beam-induced motion of individual particles, charge accumulation on the specimen induced by the electron beam and/or chemical transformation of a specimen support, see also page 10, line 28 to page 11, line 4; page 11, line 6 to page 12, line 3; Figures 1 to 3.

Page 2, line 28 to page 3, line 26 disclose that charge accumulation and charging effects are avoided by using a metal foil, see also page 1, lines 28 to 31; page 3, lines 14 to 26; page 13, line 19 to 24; page 16, lines 22 to 24; page 16, line 31 to page 17, line 4; see also page 3, lines 8 to 12 related to the metal foil being in ohmic contact with the support. Furthermore, by means of the choice of a foil material which has an appropriate mechanical stability, any effects of mechanical distortion caused by chemical change or charge imbalance to the porous metal foil may be addressed, see page 3, line 28 to page 4, line 4; page 5, lines 28 to 31 ("minimise charging, chemical and/or other similar motion-inducing processes"). Chemical changes might be avoided by using a non-reactive metal, see page 4, lines 6 to 14; page 13, lines 26 to 29.

Page 5, line 33 to page 6, line 9 makes it clear that using a support member, support elements and a metal foil all formed from the same metal (feature (c)), stress, strain, stretching and tearing (induced by a change of temperature, e.g. in cryo-electron microscopy at liquid nitrogen temperature) are mitigated. For the board, said passage indicates that using the same metal for the support member "with no discontinuity in the thermal expansion coefficient" (page 13, lines 14 to 17), the support elements and the metal foil avoids an undesired deformation of the support member itself.

Page 16, lines 24 to 29 and page 17, lines 6 to 14 disclose that the so-called "bee-swarm effect" is reduced when using "an ultra-stable substrate" and when "sample/specimen charging" is reduced. For the board, "ultra-stable" refers to a support "with no discontinuity in the thermal expansion coefficient", i.e. in accordance with feature (c), and the reduced charging is related to the electrical conductivity of the metal used, as discussed before. Page 17, lines 9 to 12 states that "[d]ecreased particle motion may be a result of, for example: high mechanical stability, reduced force on the ice due to reduced charge build-up, and elimination of chemical changes in the support which would induce stresses in a perforated foil membrane".

In the board's view, according to the application as originally filed, all these effects are related to a reduced deformation of the support member with the metal foil due to the use of a same metal for all its parts, see also e.g. Figures 8b and 9d. The application is related to the issue also mentioned on page 81 of document D1, i.e. an undesired deformation of the support member and the metal foil during cooling.

The application also mentions electron beam-induced motion of individual particles, see Figure 1 and page 10, line 35 to page 11, line 4, when the energy of the electrons is imparted to the protein samples. According to the application, this problem is reduced when using a conductive metal sample instead of a carbon sample.

The application is silent about any undesired particle motion due to the difference in thermal expansion coefficients between the vitreous ice layer and the support, as discussed in D7 (according to the appellant). The application does not relate to "additional stresses" formed in the ice sheet as allegedly shown in post-published document D9. The application is also silent about any effect on the particle motion resulting from the use of a graphene layer.

8.2.3 In view of the observations made in sections 8.2.1 and 8.2.2 above, the board is of the opinion that no synergistic effect resulting from features (c) and (f) is disclosed in or derivable from the application and that said alleged synergistic effect (possibly described in D7) is not related to the technical problem initially suggested in the application as filed.

While the present invention relates to the mechanical stability of the metal support member with its porous metal foil and its (optional) graphene layer, D7 describes a different invention, namely the reduction of undesired particle motion resulting from a difference in TEC between the support and the vitreous ice layer by using a functionalized graphene layer.

It can thus be said that the alleged synergistic effect does "alter the character of the invention" and said effect is not hinted at in the application or foreshadowed in the application.

As a side remark, the board has doubts whether the alleged synergistic effect was mentioned in document D11. For example, the page dated 26 October 2012 describes a support with feature (c). It is indicated that it can also "attach to graphene", but no method steps to add a graphene layer were provided. The page dated 8 November 2012 shows an experimental micrograph. It can be left unanswered whether AuAuGr stands for AuAu-grid or for AuAuGraphene, because, in any case, no particular synergistic effect provided by the gold support and a graphene layer is mentioned, see also D10. At most, the micrograph in D11 shows that a gold sample holder with a graphene layer indicates a motion of less 2 to 3 pixels, which is higher that the results shown e.g. in Figure 6 of the application for a substrate without a graphene layer.

8.2.4 The appellant argued that the broadest technical teaching was indicated on page 1, lines 16 to 36 of the application as originally filed.

For the reasons given before, it is questionable whether the alleged technical effect was "conceptionally comprised" by the teaching of said passage or relevant for an EM sample grid having a porous metal foil and a support member, as it is not mentioned at all in the application or derivable therefrom.

8.2.5 In view of sections 8.2.1 to 8.2.4, it is doubtful, in the present case, whether a skilled person, having the common general knowledge in mind, and based on the application as originally filed, would derive the alleged effect as being encompassed by the technical teaching and embodied by the same originally disclosed invention (G 2/21, point 2. of the Order).

However, the board is of the opinion that the question whether the requirements of point 2. of the order of G 2/21 are met can be left unanswered. In fact, the alleged technical effect, even if it were evidenced by D7 and "encompassed by the technical teaching and embodied by the same originally disclosed invention", is not achieved over the whole scope of claim 1 as will be shown in the following.

8.2.6 Turning back to post-published document D7, the board notes that this document only describes a cryo-electron microscopy sample holder having the structure shown in Figure S12. This was confirmed by the appellant during the oral proceedings before the board.

In particular, the metal used for both the support member and the metal foil in D7 is gold, the foil is 50 nm thick, the ice layer with embedded ribsomes particles is about 30 nm thick, see the text below Figures 1 and S12 of D7. According to the appellant's explanation during the oral proceedings, the diameter of the pores is about 1,2 µm, which is close the value used e.g. in Figure 5c of the application.

Furthermore, the monolayer graphene used in D7 is covalently functionalized, see section "Design and Production of Multifunctional Graphene Supports" on pages 11718 to 11720, Figure 1 and section "Graphene Functionalization" on page 11722. A reduction in particle movement is measured only for amylamine-functionalized graphene on gold support, see sections "High-Resolution Structure Determination on Multifunctional Graphene Supports" and "Discussion" and Figure S14 of the supporting information. The paragraph below the abstract of D7 also makes it clear that a graphene layer of any type is not necessarily suitable to be used as a sample support ("Pristine graphene, due to its hydrophobicity, is not a suitable substrate for preparing cryoEM specimens [...]. Still, partially hydrogenated graphene provides only a single type of adherent surface, which is not sufficient for all possible specimens"). While the appellant argued during oral proceeding that the functionalization of the graphene layer is not important for the alleged technical effect, the board notes that D7, page 11719, right-hand column, second full paragraph indictates "that the particles are interacting with the functionalized graphene". Hence, it cannot be excluded that the functionalization has an impact on the results shown in Figure S14 of D7.

Turning now to the wording of claim 1, feature (f) only requires that the graphene layer is a part of the claimed support and that it is in ohmic contact with the metal foil. No electrical or physical contact between the graphene layer and the support member or the support elements is claimed. The wording of the claim leaves it open where the graphene layer is positioned within the sample support. In particular, it is not required that the graphene layer is configured to extend across pores in said porous region of said metal foil, is configured to support said received or a further electron microscopy sample or is exposed to an electron beam. Moreover, feature (f) does not exclude that the ohmic contact is provided by additional conductive elements between the metal foil and the graphene layer. According to the description of the application, page 14, lines 35 and 36, a graphene layer might be arranged on top of the porous metal foil, which is in accordance with the arrangement according to D7, see also page 15, lines 10 to 16 of the description of the application. However, the graphene layer may alternatively be arranged "to sit between the support grid and the perforated metal foil", see page 14, lines 36 and 37 of the description of the application, or "beneath the metal foil", see page 15, line 18 to 21. Both arrangements are different from the one shown in Figure S12 of D7. During oral proceedings, the appellant confirmed that the wording of claim 1 encompasses embodiments with the graphene layer not covering any of the pores of the porous metal layer and thus neither supporting the vitreous ice layer nor being exposed to the electron beam.

In view of these observations, it must be doubted whether the graphene layer according to the broad wording of feature (f) would have any impact on an undesired particle motion or on the sample stability during cryo-EM imaging.

In addition, the wording of claim 1 does not specify the thickness of the gold layer or the size of the holes, while the application itself states that both parameters have an impact on the mechanical properties of the porous metal layer, see page 3, lines 28 to 35; page 14, lines 1 to 30. As a side remark, the important role of the metal foil thickness and the pore diameter is also discussed in post-published document D9, see page 3, second and third paragraphs.

Finally, the present application is completely silent about a functionalization of the graphene layer or the type of graphene layer used, while a functionalization is necessary to obtain the results shown in Figure S14 of D7.

Hence, the board is not convinced that the effect of reduced particle motion due to a graphene layer described in document D7 of 2019 (and further explained in D9 in 2021) is also obtained by the present invention filed in 2014 with a priority date in 2013. The teaching of D7 is not necessarily applicable to the support according to claim 1 and the supports disclosed in the application as originally filed. Hence, neither distinguishing features (c) and (f) in combination or feature (c) alone necessarily provide a solution to the technical problem resulting from the difference in thermal expansion coefficients between the vitreous ice layer and the metal foil by the alleged technical effect.

In other words, the wording of claim 1 encompasses embodiments for which it is unlikely that the technical effect shown by D7 is actually achieved. Consequently, it is not justified to reformulate the objective technical problem based on the synergistic effect allegedly provided by features (c) and (f). The appellant's formulation of the objective technical is not appropriate.

Claim 1 includes not more than an aggregation of distinguishing technical features (c) and (f). For each of them, an associated partial objective technical problem is to be formulated, as did the examining division.

8.3 As pointed out by the examining division, feature (c) provides the effect of mitigating stress, strain, stretching or tearing in the metal foil, i.e. it allows to avoid a deformation induced by a change in temperature. The objective technical problem is thus to to achieve this effect.

D1 does not disclose the material of the electron microscopy grid. The skilled person has to select a suitable EM grid when putting into practice the teaching of D1.

A skilled person using its common general knowledge knows that an undesired deformation of the support (including the metal foil) of D1 is avoided when its components have a similar coefficient of thermal expansion. This observation alone would motivate the skilled person to use the same metal (i.e. gold) for both the metal foil and the support member of D1.

As also pointed out by the examining division, D1 mentions that a flat support (i.e. a support without any deformation during cooling) is required for obtaining high-resolution electron microscopy data, see page 81, left-hand column, where D1 states that a difference in thermal expansion should be avoided. As stated by the appellant, this passage refers to a hypothetical supergrid made of conductive polymeric material doped with metal ions. Nevertheless, the skilled person would also consider this aspect when selecting a suitable material for the EM grid of D1 for supporting the gold foil. Hence, the passage of page 81 is a further motivation to use gold for the support member for supporting the gold foil disclosed in D1.

Moreover, as indicated by the examining division, the use of TEM grids made of gold were already common at the priority date, see paragraphs [0023], [0059] and [0074] of D3 and the abstract of D2 ("standard mixed-mesh Au TEM grids"). Insofar the board does not share the appellant's view that there was the preconception that an amorphous carbon sample support had to be used.

Thus, an inventive step based on feature (c) cannot be acknowledged.

8.4 Regarding distinguishing feature (f), in view of the broad wording of claim 1, feature (f) merely provides additional structural and mechanical stability. The objective technical problem associated to feature (f) is to provide this effect. Other effects mentioned by the examining division in the impugned decision (e.g. surrounding or enclosing the sample) might only be obtained by features that are not claimed.

The board shares the examining division's view that document D3 discloses the transfer of a graphene sheet to an Au Quantfoil grid with a holey amorphous carbon mesh. Paragraph [0070] of D3 presents graphene as the thinnest, strongest continuous material known and also points out that it is a "good electrical conductor", which "displays minimal charging effects from the electron beam".

The skilled person person wishing to provide additional structural and mechanical stability to the support of D1 would use the teaching of D3 and provide a graphene layer on the holey gold foil of D1. When doing so, an ohmic contact between the gold and the graphene is automatically obtained.

Under the assumption that the graphene layer extends across pores in said porous region of said metal foil and is configured to support said received electron microscopy sample, document D3 discloses in paragraph [0070] that the graphene layer is used "to span the hole with a very thin membrane" in case of a very small sample (smaller than the pores in the porous metal foil). This graphene membrane then supports the sample. This is a further motivation to add a graphene membrane to the gold foil of D1.

Thus, an inventive step based on feature (f) cannot be acknowledged, either.

8.5 In view of the above the subject-matter of claim 1 of the main request does not involve an inventive step (Articles 52(1) and 56 EPC).

9. First to third auxiliary requests

The board notes that in D1 a "cryo-EM sample support" is disclosed. Furthermore, gold is mentioned as the metal material of the metal foil.

Hence, for the reasons given for the main request, the subject-matter of respective claim 1 of the first to third auxiliary requests does not involve an inventive step, either (Articles 52(1) and 56 EPC).

10. As no allowable request is on file, the appeal must fail.

## Order

## For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chairman:



S. Sánchez Chiquero T. Häusser

Decision electronically authenticated