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Datasheet for the decision of 12 February 2026

Case Number: T 0097/23 - 3.4.03

Application Number: 17731174.3

Publication Number: 3475681

IPC: G01N1/42, F25D3/11, H01J37/20, G01N1/28

Language of the proceedings: EN

Title of invention:
METHOD OF AND APPARATUS FOR PREPARING SAMPLES FOR IMAGING OR DIFFRACTION EXPERIMENTS UNDER CRYOGENIC CONDITIONS

Patent Proprietor:
Universiteit Maastricht

Opponent:
MURGITROYD & COMPANY

Headword:

Relevant legal provisions:
EPC Art. 100(b), 83

Keyword:
Grounds for opposition - insufficiency of disclosure (yes)

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Case Number: T 0097/23 - 3.4.03

D E C I S I O N
of Technical Board of Appeal 3.4.03
of 12 February 2026

Appellant: (Opponent)	MURGITROYD & COMPANY Murgitroyd House 165-169 Scotland Street Glasgow G5 8PL (GB)
Representative:	Stellbrink & Partner Patentanwälte mbB Widenmayerstrasse 10 80538 München (DE)
Respondent: (Patent Proprietor)	Universiteit Maastricht Minderbroedersberg 4-6 6211 LK Maastricht (NL)
Representative:	De Vries & Metman Overschiestraat 180 1062 XK Amsterdam (NL)
Decision under appeal:	**Decision of the Opposition Division of the European Patent Office posted on 18 November 2022 rejecting the opposition filed against European patent No. 3475681 pursuant to Article 101(2) EPC.**

Composition of the Board:

Chairman	T. Häusser
Members:	M. Papastefanou
	E. Mille

Summary of Facts and Submissions

I. The appeal of the opponent is against the decision of the opposition division rejecting the opposition against the European patent EP 3 475 681 B1.

The opposition was based on the grounds for opposition under Article 100(b) EPC (insufficient disclosure) and 100(a) EPC (lack of novelty and inventive step).

II. The appellant opponent ("opponent") requested that the decision under appeal be set aside and the patent be revoked in its entirety.

The respondent patent proprietor ("proprietor") requested that the appeal be dismissed. As an auxiliary measure, it requested that the patent be maintained according to one of the 1st to 6th auxiliary requests filed with its reply to the statement setting out the grounds of appeal, or one of auxiliary requests 3A to 3D and 4A, filed with its letter dated 9 January 2026.

III. Reference is made to the following document:

D11: *Heat transfer due to a round jet impinging normal to a flat surface*, A. K. Mohanty and A. A. Tawfek, International Journal of Heat Mass Transfer, Vol. 36, No 6, pp. 1639-1647, 1993.

IV. **Claim 1 as granted** has the following wording (features' numbering as in the impugned decision; reference signs are omitted here and below):

[1.0] *Method of preparing a sample for imaging under cryogenic conditions or diffraction experiments*

under cryogenic conditions, comprising the steps of

[1.1] *applying a sample to a sample carrier, in particular a grid, the sample carrier comprising a film on a support and*

[1.2] *vitrifying the sample,*

[1.2.1] *wherein the sample is vitrified by directing a jet of liquid coolant to the center of the sample carrier and onto the sample so that the sample is vitrified first in the center of the support and from there towards the edge of the support.*

V. The detailed wording of the auxiliary requests is not relevant. Claim 1 of all the auxiliary requests 1 to 6, 3A to 3C and 4A includes the last part of feature [1.2.1]:

"*... so that the sample is vitrified first in the center of the support and from there towards the edge of the support.*"

VI. In addition, in claim 1 of **auxiliary request 1**, feature [1.1] has been amended as follows (amendments marked by the board):
"*applying a sample to an EM grid ~~a sample carrier, in particular a grid~~, the ~~sample carrier~~ grid comprising a film on a support and*".

Feature [1.2.1] has been amended correspondingly (amendments marked by the board):
"*wherein the sample is vitrified by directing a jet of liquid coolant to the center of the grid ~~sample carrier~~ and onto the sample ...*".

VII. In claim 1 of **auxiliary request 2**, in addition to the amendments carried out in claim 1 of auxiliary request

1, the following feature has been added at the end:

"*wherein the velocity of the at least one jet of coolant is in a range from 1 to 50 m/s and wherein the combined mass flow rate of the jets is in a range from 0.1 to 100 L/min.*"

VIII. The relevant arguments of the parties can be summarised as follows:

- The opponent argued that the patent did not provide sufficient information to the skilled person to carry out the claimed method. The value ranges for the velocity and the flow rate of the liquid coolant jets were too broad. The value ranges for the diameter of the nozzles of the liquid coolant jets that could be derived from them was equally broad. The skilled person had to carry out extensive experiments to determine which combinations of those values would achieve the desired technical effect of the vitrification starting from the center of the sample. This represented undue burden.

- The proprietor argued that the skilled person would be aware of certain inherent characteristics of the liquid coolant jets from common general knowledge. The skilled person would also, based on its common general knowledge and the disclosure of the patent, be able to determine the appropriate combinations of parameters in order to achieve the desired vitrification. Moreover, the sample being vitrified first in the center of the support and from there towards the edge of the support was to be understood as a functional feature of the claimed method and not merely as a technical effect.

Reasons for the Decision

The invention

1. The invention relates to a method and an apparatus for preparing samples, such as particles, macromolecular compounds (e.g. proteins, viruses, cells, bacteria), nanoparticles and crystals for imaging (e.g. in a cryo-electron microscope (EM)), or diffraction experiments (e.g. inspections by ionizing radiation), under cryogenic conditions (see paragraph [0001] of the opposed patent).

2. The preparation of the samples consists in their vitrification. "Vitrification" in the present context is to be understood as rapid freezing (cooling rates "in excess of 50.000 K/s", see paragraph [0010]). The idea is that the sample is frozen so quickly that no ice crystals are generated and it turns into an amorphous solid (like glass). This is achieved by spraying the sample with a liquid coolant at very low temperature and high velocity.

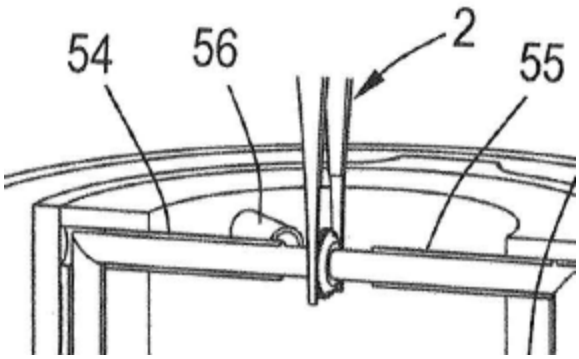
3. The present invention aims at achieving vitrification of the sample from the center towards the edges. This is achieved by aiming a jet of liquid coolant to the centre of the support on which the sample is applied. In this way, the sample is vitrified first in the center and then towards the edge(s) of the support. As the sample vitrifies before the (effect of) liquid coolant reaches the edge(s) of the support, any influence caused by the vitrification of the edge(s) of the support on the sample is excluded or at least reduced (see paragraphs [0008] and [0009]).

Patent as granted, sufficiency of disclosure (Article 100(b) EPC)

4. The contested point relates to feature [1.2.1] of claim 1, and in particular to the last part of that feature:

"so that the sample is vitrified first in the center of the support and from there towards the edge of the support".

5. For ease of understanding, the relevant part of Figure 5 of the patent, which represents an embodiment of the apparatus used in the claimed method is reproduced below.



6. In this figure, a holder (2) holds the sample carrier carrying the sample on a support between the nozzles (ends) of two pipes (54, 55). Each of the nozzles directs a jet of liquid coolant to the center of the sample carrier to vitrify the sample. It is to be noted that in the method of granted claim 1 one jet of liquid coolant is defined.

7. The disputed feature is understood as a technical effect of the claimed method. In other words, it is expected that when a skilled person carries out the other steps of the claimed method, they may (when the relevant parameters are selected accordingly) achieve

that the sample is vitrified first in the center of the support and from there towards the edge of the support. As the technical effect of the method is included in the claim, in order to meet the requirement of sufficiency of disclosure, the patent must provide sufficiently clear and complete information to the skilled person to achieve this technical effect.

8. It is common ground that in carrying out the claimed method, several aspects of the corresponding technical setup must be determined. For example, the skilled person must determine the appropriate diameter of the nozzle(s), the diameter of the sample support, the distance between the sample support and the nozzle, the velocity and the flow rate of the jet of liquid coolant. The patent does not provide much information related to those aspects.

9. According to feature [1.2.1] of claim 1, the jet of the liquid coolant is directed to the center of the sample support. It is uncontested by the parties that this is not sufficient to achieve the desired result. By looking at Figure 5, the skilled reader would readily understand that other factors such as the distance between the nozzle and the sample support, the relation between the diameters of the nozzle and the sample support (i.e. which one is greater than the other), and the velocity of the jet, also influence the way the jet of liquid coolant impinges on the sample support and, consequently, the way the sample is vitrified.

10. Regarding the diameter of the sample support, the patent does not contain any information other than one example mentioning the support being a normal/standard EM grid (paragraphs [0039] and [0066] of the specification). It is considered common general

knowledge that such a grid has a diameter of 3.05 mm (see paragraph [0007] of the patent specification).

11. It is undisputed that there is no disclosure of any value for the diameter of the nozzle(s) in the patent. In paragraph [0014] and claim 5 there are ranges of values for the velocity and the flow rate of the jets: 1 to 50 m/s (preferably 1 to 20m/s) for the velocity and 0.1 to 100 L/min for the flow rate. From the combination of the velocity and the flow rate of the jet, the diameter of the corresponding nozzle can be derived using only common general knowledge. On the basis of the ranges given above, the corresponding range of possible diameters for the jet nozzles is 0.14 to 32.6 mm. This is common ground between the parties. Compared to a sample support with a diameter of 3.05 mm, it is evident that not all the nozzles with a diameter within that range would achieve the desired vitrification. This was also acknowledged by the proprietor.

12. Regarding the distance between the nozzle and the sample support, there is no relevant suggestion or indication in the patent. According to the opponent, for a given jet velocity the vitrification result would depend on the distance which the liquid coolant jet had to "travel" before impinging on the sample support. The combination of the jet velocity and the distance to the sample support has to be such that the jet of liquid coolant can keep its horizontal form so it can impinge on the center of the sample support. The proprietor argued that the term "jet" had a specific meaning in the technical context of the patent and that the skilled person would know, for example, that a jet of liquid had to be "formed" after it was ejected from the nozzle. A certain minimum distance to the sample

support was therefore necessary. The proprietor referred to document D11, which it argued represented the skilled person's common general knowledge, and to the examples of distances between the nozzle and the sample support ("plate" in D11), see page 1639, bottom of the left column and bottom of the right column. The skilled person was therefore in a position to determine the appropriate distance between the sample support and the jet nozzle using only their common general knowledge.

13. The proprietor also referred to Figure 5 of the patent and argued that the skilled person would understand from that figure that the diameter of the liquid coolant jet was smaller than the diameter of the sample support (see the part of Figure 5 shown in point 5. above). At the same time, the proprietor argued that the desired technical effect could also be achieved by jets with a slightly larger diameter than the sample support. If the liquid coolant jet "travelled" a sufficient distance before impinging on the sample support, the liquid at the periphery of the jet (imagining the liquid jet as a cylinder) would travel less fast than the liquid in the center of the jet (e.g. due to resistance of the air) so that the part of the liquid jet impinging on the sample support would have a smaller diameter than the liquid jet as a whole. It would thus still be possible to vitrify first the center of the sample with a liquid coolant jet of a greater diameter than the sample support.

14. Summarising, the proprietor argued that the skilled person was aware that the use of the term "jet" implied some inherent characteristics of the jet of liquid coolant defined in claim 1. Although it was clear to the skilled person that not all the jets with diameters

within the range 0.14 to 32.6 mm would achieve the desired verification, the skilled person would be in a position to select the appropriate diameters within that range without undue burden. Even if it were accepted that Figure 5 was only schematic and that particular dimensions or scales could not be derived from it, the skilled person looking at it would recognise that the diameter of the liquid jet was smaller than the diameter of the sample support. This was a limitation that would reduce the burden of the skilled person when selecting the appropriate jet diameters from the available range.

15. The board is not convinced by the proprietor's arguments.

16. Assuming that the skilled person is aware of any inherent characteristics of a "jet", the board cannot see how this would help them sufficiently in the present context. D11 provides some indications about possible distances between the nozzle and the impinging point of the jet on the sample support. Combining the disclosures in the various examples mentioned in D11, the distance between the nozzle and sample support could be between 2 and 20 times the diameter of the nozzle. Even if the other conditions about the various applications in D11 were ignored and this range was taken as teaching for the present context, the skilled person would still have to identify which values within this range of distances would be suitable for carrying out the claimed method. In addition, the suitable distance has to be determined in view of a combination of other parameters, in particular the velocity of the jet and the diameters of the nozzles.

17. Summarising, the skilled person is faced with the task to determine appropriate combinations of the following parameters within their corresponding value ranges:
- diameter of the sample support; the patent provides only one example of 3.05 mm;
- diameter for the jet nozzle; the patent implicitly discloses a range of 0.14 to 32.6 mm;
- distance between the nozzle and the sample support; 2 to 20 times the nozzle diameter as indicated in D11;
- jet velocity: 1 to 50 m/s, preferably 1 to 20 m/s; as indicated in the patent
- jet flow rate: 0.1 to 100 L/min as indicated in the patent (this parameter is related to the nozzle diameter and the jet velocity).

18. It is also noted at this point that the proprietor's argument that the skilled person would derive from Figure 5 that the diameter of the liquid jet coolant is smaller than the diameter of the sample support is contradicted by its argument that also jets with greater diameter than the sample support can achieve the desired vitrification (see point 13. above). This leads to the conclusion that it is not implicit or inherent in the present context that the diameter of the liquid coolant jet is smaller than the diameter of the sample support.

19. The skilled person is thus faced with the task of determining the combinations of values for these parameters which would achieve the result that the sample is vitrified first in the center of the support and from there towards to the edge of the support according to feature [1.2.1] of claim 1. In the board's view, this requires extensive experimentation which goes beyond what would be considered an acceptable

burden in the context of the assessment of the sufficiency of the disclosure.

20. The proprietor also argued that the disputed feature of claim 1 was to be understood as a functional feature of the claimed method and not as a technical effect achieved by it. In other words, any method that would not produce the desired vitrification starting from the center of the sample was not included in the claimed scope. Accordingly, any embodiments within the value ranges of the various parameters which did not achieve the desired vitrification were not to be considered in the question of sufficiency of disclosure because they were not part of the claimed invention.

21. The board cannot see how considering the disputed feature a functional feature of the claimed method could lead to a different conclusion regarding sufficiency of disclosure. Even in such a context, the skilled person would still be faced with the task of identifying which of all the potential embodiments falling within the value ranges of the various parameters achieve a vitrification starting from the center of the sample. Whether this is considered a technical effect or a functional feature does not change the fact that the skilled person must carry out extensive experimentation involving undue burden.

22. The board's conclusion is therefore that claim 1 as granted does not disclose the invention in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art. Accordingly, the ground for opposition under Article 100(b) EPC prejudices the maintenance of the patent as granted.

Auxiliary requests

23. Claim 1 of all auxiliary requests 1 to 6, 3A to 3C and 4A comprises the disputed feature (last part of feature [1.2.1]).

24. The proprietor pointed to auxiliary requests 1 and 2, which included additional limitations regarding the sample support (being an EM grid; auxiliary request 1), and additionally the velocity and the flow rate of the jet (auxiliary request 2). The proprietor argued that these limitations limited the claimed scope and provided the skilled person with sufficient information to be able to select the appropriate embodiments of the claimed method without undue burden.

25. The board does not agree. Claim 1 of auxiliary request 1 defines that the sample support is an EM grid. Claim 1 of auxiliary request 2 includes in addition the value ranges for the velocity and the flow rate of the jet(s) from paragraph [0014] of the patent. When assessing the sufficiency of disclosure in the granted patent, the entire disclosure of the patent was considered. In view of the fact that claim 1 as granted does not contain any information about any of the parameters regarding the claimed method, these limitations in the description were indeed taken into account. In the present context, therefore, whether these limitations are only described in the description or are also included in the claims does not make any difference that could lead to a different conclusion regarding the assessment of sufficiency of the disclosure.

26. Consequently, the board concludes that none of the auxiliary requests 1 to 6, 3A to 3C and 4A discloses the claimed invention in a sufficiently clear and complete manner for the skilled person to carry it out,

contrary to the requirements of Article 83 EPC.

27. As none of the requests of file is allowable, the patent cannot be maintained according to any of the pending requests. The question of admittance of the auxiliary requests into the proceedings can be left open.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The patent is revoked.

The Registrar: The Chairman:

S. Sánchez Chiquero T. Häusser

Decision electronically authenticated