



Scientific Misconduct

The Strange Case of a Gel and its True Origins

Years ago, suspicions arose that the image of a protein gel, published in a *Proteomics* article, had been plagiarised. An internal investigation by the journal itself, however, rejected the incriminations as unsubstantiated. Now, this mysterious case has again come to a head. Apparently, the image was indeed manipulated, after all.

In February 2005, *Proteomics* published the article “A proteomic approach for investigation of photosynthetic apparatus in plants” (vol. 5(3): 746-57). Lead author is the former PhD student Corrado Ciambella, senior author his supervisor Lello Zolla from the University of Tuscia in Viterbo/Italy; further co-authors are professors Eva-Maria Aro from the University of Turku and Peter Roepstorff from the University of South Denmark in Odense. In figure 4 of the article, the authors present the scan of a two-dimensional Blue Native/SDS (2D-BN/SDS) gel run with photosynthetic proteins from barley thylakoid membranes. The single spots of the separated proteins are labelled on the scan according to their identification by subsequent mass spectrometry.

Shortly thereafter, Bernhard Granvogel, then PhD student at the botany department in the University of Munich, comes across the gel scan – and it stops him in his tracks. The pattern of the spots promptly points him to a 2D-BN/SDS gel that he himself had received, also with solubilised thyla-

koid membrane proteins from barley, three years earlier. Back then, he, together with his supervisor, Lutz Eichacker, had submitted a manuscript for publication to *Proteomics* – including said gel. Despite submitting a second revised version, the reviewers had rejected it twice. A third attempt to publish it in *Biochemistry* had also failed.

Puzzled, Granvogel digs his gel scan out of the depths of his computer for comparison. Now, he and Eichacker are no longer in any doubt: Figure 4 in Ciambella *et al.* originated from their own gel.

Figure 4 originated from their own gel

Eichacker asks his former fellow student and patent attorney Michael Fleuchaus, to check this “issue”. Fleuchaus remembers:

“Given that the data I received at the time were correct (and there is no reason to doubt it), I came to the preliminary conclusion that Figure 4 in Ciambella *et al.* with almost absolute certainty originated through unauthorized use of the image from the original gel by Granvogel/Eichacker – and, possibly, in addition, by faking results.”

Fleuchaus advises Eichacker to take civil action against the authors of Ciambella *et al.* Eichacker, however, decides not to do so. He prefers a less confrontational solution with the authors and *Proteomics*. Therefore, he informs *Proteomics*' Chief Editor Michael Dunn and the responsible managing editor at Wiley VCH, Hans Joachim Kraus, about the suspicion of manipulation. Their subsequent actions at *Proteomics* are described by Michael Dunn as follows:

“I therefore wrote to Professor Lello Zolla, the senior author of the Ciambella *et al.* paper, on 20th July 2005, asking him to comment on this situation. After several rounds of correspondence with Prof. Zolla, it became clear that the blue native 2-DG work was carried out by Dr. Ciambella in collaboration with Prof. Eva Maria Aro (University of Turku, Finland), an acknowledged expert in this field. The mass spectrometry of the proteins of interest was carried out by Prof. Peter Roepstorff (University of Southern Denmark), an internationally recognised expert in MS. I therefore wrote to both professors Aro and Roepstorff for their response to the situation.”

Zolla, Aro and Roepstorff admit striking similarities in both gels but also emphasise clear differences. Roepstorff, for example, writes:

“The gel in our paper, although similar, has definite (but small) differences from the unpublished gel of the Eichacker group. Therefore, I feel confident that the data presented in the Ciambella paper are original.”

Kraus and Dunn agree with this judgement. They inform Eichacker, who, according to Dunn, suddenly accepts the results of the internal investigation and consents not to follow this issue any further. Instead, Eichacker asks whether he might re-submit the twice-rejected Granvogl manuscript, inclusive of the implied gel, in its revised form to *Proteomics*. Dunn agrees but he clarifies:

*“Please note that this was a ‘request’ to submit from Dr Eichacker, not an ‘invitation’ to submit from our journal. We agreed to this request, and the paper (Granvogl et al.) was submitted online on 22nd December 2005. It was processed and handled in the normal way, involving full peer review. The paper was eventually published in *Proteomics* in 2006.”*

Eichacker, a Professor in the Centre for Organelle Research (CORE) at the Stavanger University in Norway since 2008, however, has a slightly different view, in retrospect:

*“Mister Dunn offered to review a revised version of my work from 2002 again and to publish it after completion of the review process. I agreed under the condition that the data were published together with the original date of the first submission. *Proteomics* fulfilled that by mentioning this date appropriately. Accordingly, there is now the following footnote in the article of Granvogl et al. (*Proteomics* 6(12): 3681-95): ‘The work in this article was originally submitted to *Proteomics* on November 1, 2002.’”*

Why did Eichacker give in?

The question, nevertheless, remains as to why Eichacker suddenly gave in, despite still being convinced of the “gel-robbery”. Eichacker states:

“I was certainly happy about this offer of Mr Dunn [...]. My prior aim was to find a way to secure the publication for my PhD student, which was important for his thesis at that time.”

Even if not completely satisfying, the issue seemed, nevertheless, closed. Why then, has it blown up again, now - out of the blue and years later?

Last year, *Lab Times* received an anonymous email saying, amongst other things:

“[...] would like to encourage you to discuss a recently published, clearly plagiarised and retouched image as a ‘cautionary tale’ in your journal and maybe get in touch with the authors and the publishers. I know from a rather secure source that Fig. 1 (incl. data to Proteomics) in Granvogl et al. is the original and Fig. 4 in Ciambella et al. (although published first) is a ‘pirate copy’. See for yourself!”

Obviously, someone wasn't exactly satisfied with the treatment of this case so far. And rightly so, as the subsequent enquiries were to show.

The issue, however, still exists

Lab Times contacted eight independent 2D-gel-specialists asking for an expert opinion on both gels. Four did not answer at all and two were very evasive. Hans-Peter Braun from the Institute of Plant Genetics from Hannover University, on the other hand, was rather more precise:

*“Although there are clear differences in some areas of the gel, the protein spots in other areas are remarkably similar. Especially gel ‘disorders’, e.g. small air bubbles, are on identical positions on both gels. Therefore, I do not doubt that the identical gel was used for both figures. The Ciambella et al. publication is older (2005) but the Granvogl et al. publication from 2006 states on the title page that this manuscript had already been submitted for the first time to *Proteomics* in 2002. If that original version of the manuscript already contained this protein gel, the suspicion is obvious that it was (pirate) copied and manipulated for Ciambella et al. 2005. [...] I cannot understand why *Proteomics*, if the suspicions are true (and I am convinced of it), still hasn't officially retracted the Ciambella et al. publication.”*

Thierry Rabilloud, proteomics specialist at the CEA (Centre of Atomic Energy) in Grenoble and notably Associate Editor at *Proteomics*, also replied to our query, even if at first moodily:

“I cannot say that it will be my pleasure but it will be my duty to perform such an analysis for you.”

For this analysis, Rabilloud was furnished with the original articles, various versions of the Granvogl et al. manuscript as well as with all original documents from Granvogl and Eichacker – including a scan of the bare original gel without any labels. For the Ciambella et al. paper no original data and images could be obtained, neither from the authors nor from *Proteomics*.

Proteomics Chief Editor Mike Dunn explained:

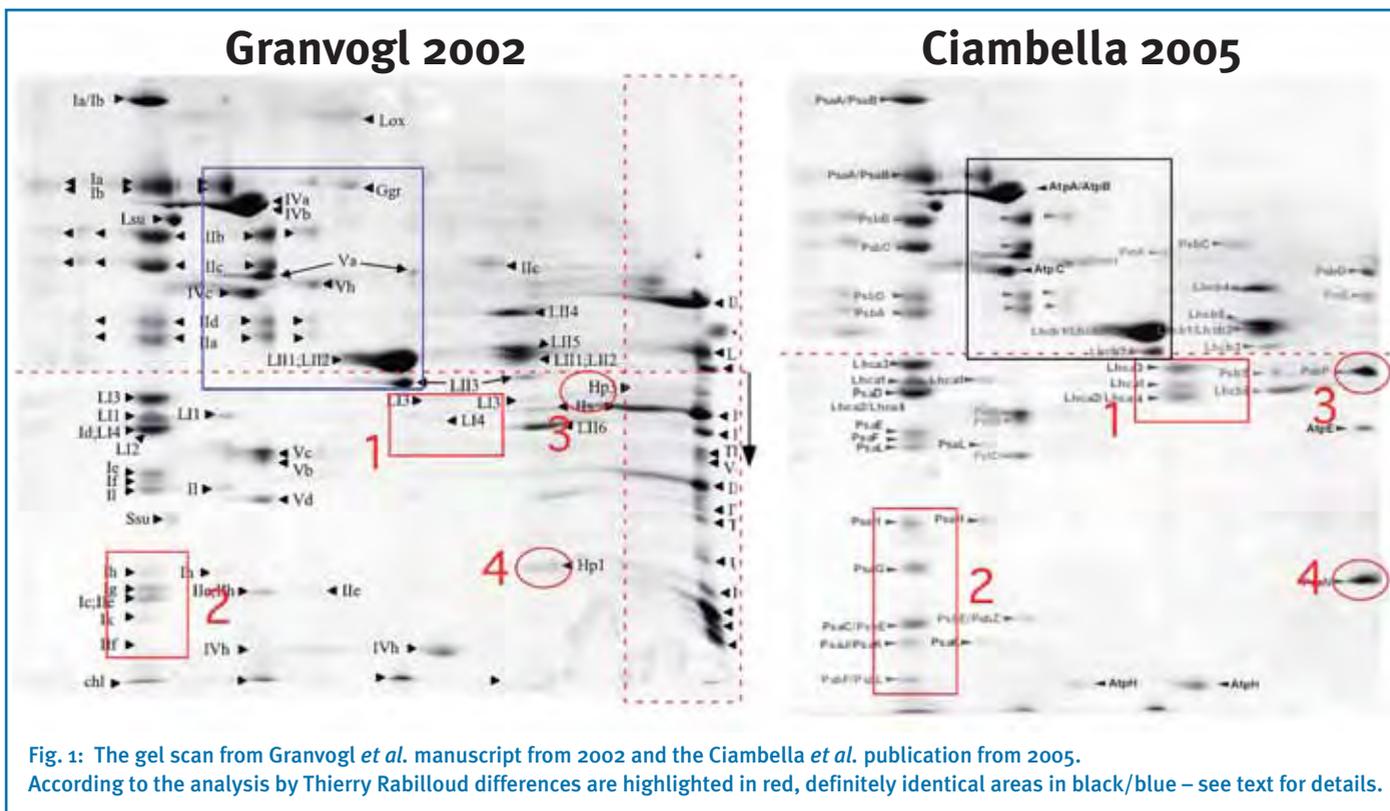


Fig. 1: The gel scan from Granvogl *et al.* manuscript from 2002 and the Ciambella *et al.* publication from 2005. According to the analysis by Thierry Rabilloud differences are highlighted in red, definitely identical areas in black/blue – see text for details.

“I am afraid that I no longer have access to the primary data for the Ciambella *et al.* manuscript as mentioned by Professor Zolla. You may be aware that I retired from my full-time academic position at University College Dublin (Ireland) at the end of October 2009. [...] There was no way that all of this archival material could be taken into my family home in London, which necessitated disposal of the majority of this material, including the primary data for the Ciambella *et al.* manuscript.”

Where is the first author?

Lello Zolla, Ciambella’s supervisor and corresponding author of the paper, declared:

“Prof. Michael Dunn contacted me following Prof. Eichacker’s request and he asked me for all the original data of our submission. At that time [2005, the ed.], I was able to provide both him and the Editorial staff of the journal with a CD contain-



Lello Zolla:
“It’s impossible for me to recover the original material.”

ing a plethora of gel images obtained from my PhD student, along with the mass spectrometry identifications of all the spots performed in Peter Roepstorff’s lab. Now it’s impossible for me to recover this material because it has been a long time since Dr. Cor-

rado Ciambella stopped working in my lab. [...] I do not know what he is doing and how to reach him.”

That only a few years after sending off a CD, all original data has supposedly disappeared is, in principle, a scandal in itself. Apart from that, *Lab Times* was also unable to trace first author Corrado Ciambella. Nevertheless, the available material was still sufficient for Thierry Rabilloud to perform a detailed analysis. And his results should definitely re-open this case.

Rabilloud investigated the scans of both gels by applying various image analysis software. In his final report he first stresses the obvious differences between both gels – concluding ...

“... The differences present in the lower half completely support the fact that the figures come from independent experiments and independent gels.”

Two paragraphs later, however, the report takes a sudden and sharp turn:

“This is, however, not the end of the story and at this point it becomes very puzzling. When the zone corresponding to the blue/black box in figure 1 [Shown here as Fig.1]

is extracted from the original Granvogl gel, the original Granvogl 2002 manuscript figure, the Ciambella 2005 figure and the Granvogl 2006 figure, stretched or compressed to have exactly the same size, as shown in figure 5 [Fig.2 here], it is absolutely clear that this zone is 100% superimposable in all figures, at a point that cannot be coincidental.”

Puzzling air bubbles

And further down, Rabilloud comes to one of the biggest mysteries of the whole matter:

“Where the picture becomes very puzzling lies in the detailed analysis of bubbles 1, 2 and 3 [see Fig.2]. The bubble series highlighted in circle 2 is completely superimposable between the gels [...]. However, what puzzles me most are bubbles 1 and 3. If the data submitted by Granvogl *et al.* in 2002 and 2003 were pixelated, as is a paper figure, a JPEG file or a TIFF file, it was impossible for Ciambella *et al.* to reconstruct bubbles 1 and 3 as they are present in their figure, because of the presence of annotations (arrows, arrowheads) in the Granvogl *et al.* 2002 and 2006 figures. However, bubbles 1 and 3 are exactly identical between the original, not annotated Granvogl gel and the Ciambella figure. This means in turn that Ciambella *et al.* must have had access, in one way or another, to the original not annotated Granvogl gel, i.e. a piece of information that should have not been present, by

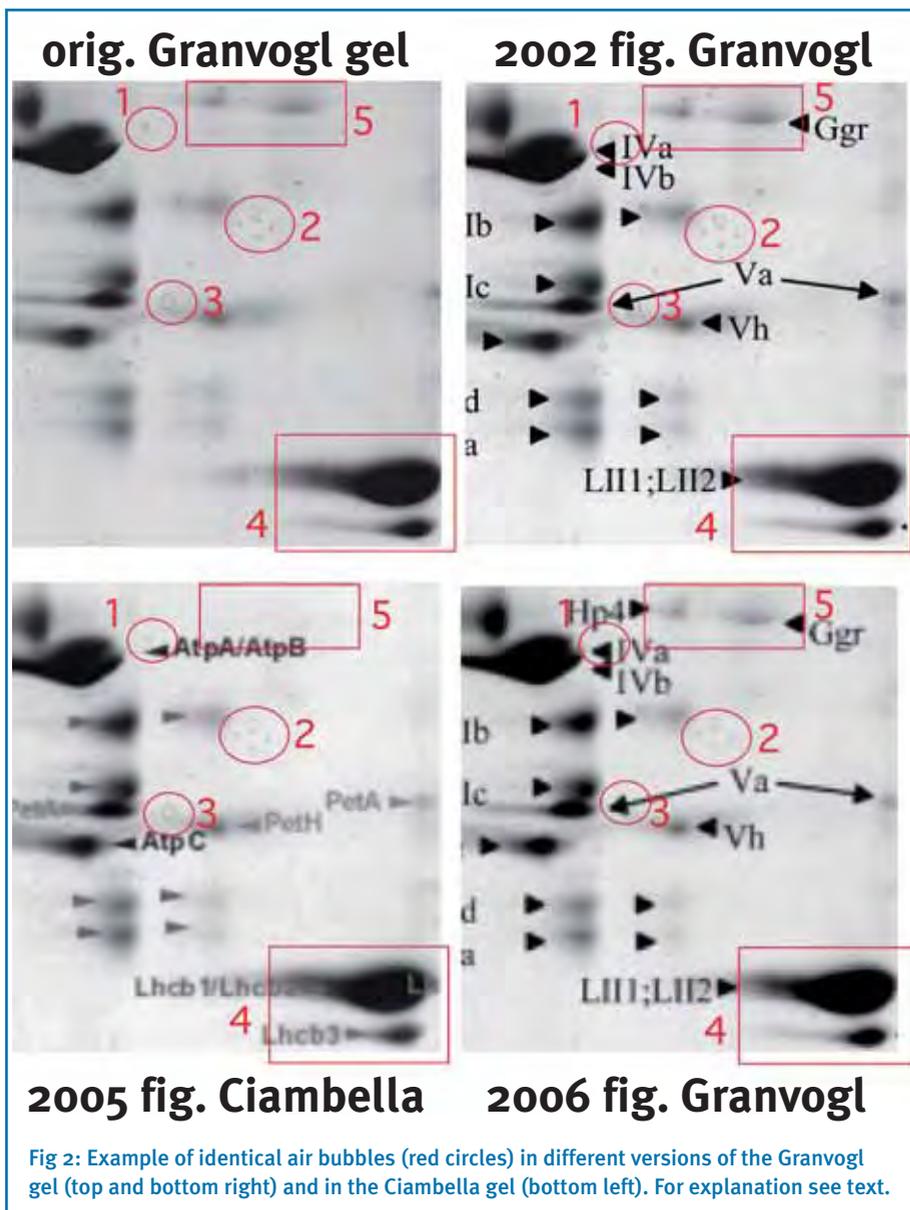


Fig 2: Example of identical air bubbles (red circles) in different versions of the Granvogl gel (top and bottom right) and in the Ciambella gel (bottom left). For explanation see text.

any means, in the manuscripts submitted in 2002 and 2003 by Eichacker's lab."

This "mystery", however, has no influence on Rabilloud's basic judgement. In bold letters he writes:

"My final conclusion is that the Ciambella figure is a composite figure, in which a part of the original, not annotated Granvogl gel, but not of any figures submitted together with a manuscript, has been pasted on an otherwise original gel. [...] Please also note that it would have been impossible to reach this conclusion on the basis of the manuscript figures only. The original gel was absolutely needed."

And what about the other data?

And, finally, Rabilloud adds another important point:

"Of course, further investigations would be required to check whether real mass spec-

trometry data were obtained for the 'copied spots' by Zolla's group in cooperation with Roepstorff's group, but this is another part of the story."

Lello Zolla's reaction is thin. He only emphasises that yes, he is the corresponding author but that he had not conducted the experiments himself. And that, after all, the 2D gels came from Eva-Maria Aro's laboratory in Turku. At the end of his response he even tried a rather fleshless counter attack:

"Carefully reading Rabilloud's report, I ask: Did Ciambella manipulate Granvogl's figures, or the contrary?"

Co-author Eva-Maria Aro, in contrast, was shocked. She wrote:

"This is very alarming! We all are very upset! We must know the truth! [...] I am furious because we feel that Lello is blaming us. My people here and I, we all feel that we were

just helping and teaching his student, Ciambella. We all have now investigated the gels very carefully and cannot ignore that the 'air bubbles' are exactly in the same places. Such a coincidence is not possible.

"Unfortunately, we do not have the original gel scans here in Turku. I am still trying to find out whether they are stored in the Centre of Biotechnology here in Turku. However, their proteomics unit where we scanned our gels at that time meanwhile has a new personnel and new computers."

Proteomics Chief Editor Mike Dunn, due to Rabilloud's investigation, now also admits that...

"[...] there was clearly serious malpractice in the Ciambella publication, although I believe that it is very difficult to understand how this could have occurred."

Another point, however, appears to be equally important for Dunn:

"Prof. Rabilloud concludes that the Ciambella figure is substantially different from the Granvogl figure, but there is evidence of malpractice which is restricted to a part of the image and that this was very difficult to detect. We believe this shows that there was no unethical behaviour on the part of Proteomics and any of the people concerned with the peer review process of the original 2002 Granvogl manuscript. Prof. Rabilloud also goes on to state that the conclusions of the internal investigation at Proteomics five years ago were therefore essentially correct."

Shock and agreement

And he suggests ...

"... to undertake further investigations to check whether real mass spectrometry protein identification data were obtained from the protein spots indicated in the figure in the Ciambella paper. Unfortunately, the head of the laboratory that performed this MS analysis, Prof. Roepstorff, has now retired and we are currently in contact with his successor, Prof. Ole Jensen, to establish the originality/validity of the MS data reported in the Ciambella paper."

Now, a whole year later, *Lab Times* has still not been informed over any results of such an investigation – whether confirming Rabilloud's conclusions or possibly rebutting it.

For Lutz Eichacker, however, those confessions no longer go far enough. Two things still bother him: first, Rabilloud's statement that, at the most, only a part of the Ciambella gel had been copied from the former Granvogl gel; and secondly, the conclusion that due to the specific labelling of the two gels and in order to carry out such a manip-

ulation, Ciambella *et al.* could only have used the unlabelled Granvogl gel – as it was hardly available from the original Granvogl *et al.* manuscript.

Eichacker thinks Rabilloud only wanted to forestall the awful suspicion that something might have gone awry with the Granvogl manuscript at *Proteomics* – possibly, that the included gel could have been illicitly passed on to Ciambella *et al.* during the review process.

Eichacker quite polemically:

“The final conclusion sounds like a farewell to the rescue of the peer review in Proteomics: Dear God, please let no reviewer unethically misappropriate a submitted manuscript ...”

And he clarifies:

“It’s all about which kind of files were actually submitted to the journals. These often were Word and PowerPoint files with integrated original scans labelled in PowerPoint. From such a file it is certainly possible to remove all arrows and labels.”

Indeed gel scans, in particular, are often labelled in PowerPoint. Accordingly, *Proteomics* explicitly does accept the submission of figures in PowerPoint format as stated in their “Instructions to Authors”.

Eichacker’s former lawyer, Michael Fleuchaus, had also come to a similar conclusion after his analysis in 2005:

“By the way, back then I had also found out that the picture of the gel ‘without labels’ could have been easily taken from the material submitted by Granvogl/Eichacker – just by using some (simple) software tricks.”

But that’s not all. Eichacker is also convinced that Ciambella *et al.* not only included part of the Granvogl gel in another gel of their own but instead plagiarised the whole gel and manipulated it by adding a few bands according to their needs. At the time, Eichacker had the gels independently checked by the company DECODON, which subjected them to their professional software for 2D gel image analysis. He writes:

“The protein analysis at DECODON [...] led to the production of two superimposable protein gels (ignoring the additionally added



Thierry Rabilloud:
“...the Ciambella figure is a composite figure...”

couple of protein spots). Independently, the air bubble inclusions were also superimposed by the same procedure.”

This result, as Eichacker writes, could also be achieved more simply in PowerPoint by superimposing both scans and slightly stretching the Ciambella gel. *Lab Times* received such PowerPoint analyses by Eichacker – and they indeed seem to confirm his point.

His conclusion, therefore, is clear: If over the whole gel protein spots can be superim-

posed and, at the same time and completely independently from that, unspecific air bubbles and smudges, which are not at all influenced by the physics of the protein separation process, also show up in exactly the same places on both gels – ...

“... then this is the end of looking at differences. Rather, we can definitely switch over to looking at identity – and, therefore, to an analysis of plagiarism and data manipulation.”

Some gnawing questions still open

Nevertheless, some mysteries still remain to be solved in this rather odd case. Was really the whole Granvogl gel copied and manipulated for Ciambella *et al.* – or indeed only part of it? If yes, how and in which form were Ciambella *et al.* able to get their hands on the Granvogl gel? Did, in the end, someone actually violate the ethical rules of peer review at *Proteomics* (or at *Biochemistry*, where the gel scan was also submitted in 2003) and “acquire” the Granvogl gel scan? And where does the mass spectrometry data for the individual protein spots in Ciambella *et al.* come from, if they apparently did not have the corresponding original gel (or, at least, part of it)?

In one crucial point, however, expert reviewer Thierry Rabilloud, *Proteomics* Chief Editor Mike Dunn and co-au-

thor Eva-Maria Aro, in the meantime, agree with Lutz Eichacker: One way or the other, figure 4 in Ciambella *et al.* is definitely manipulated.

This alone should certainly be sufficient for a retraction of the article.

RALF NEUMANN



Lutz Eichacker:
“...we can switch to an analysis of plagiarism and data manipulation...”