Special Issue: The Circulation of Images in the Life Sciences 1800-present

"Conducted properly, published incorrectly": the evolving status of gel electrophoresis images along instrumental transformations in times of reproducibility crisis

Nephtali Callaerts[a], Alexandre Hocquet[b,c] and Frédéric Wieber[b]

[a] UDC, IRDENa, Université de Namur, Namur, Belgium

[b] AHP – PreST, Université de Lorraine & CNRS, Nancy, France

[c] KHK cultures of research, RWTH, Aachen, Germany

Abstract

For the last ten years, within molecular life sciences, the reproducibility crisis discourse has been embodied as a crisis of trust in scientific images. Beyond the contentious perception of "questionable research practices" associated with a digital turn in the production of images, this paper highlights the transformations of gel electrophoresis as a family of experimental techniques. Our aim is to analyze the evolving epistemic status of generated images and its connection with a crisis of trust in images within that field.

From the 1980s to the 2000s, we identify two key innovations (precast gels and gel docs) leading to a "two-tiered" gel electrophoresis with different standardization procedures, different epistemic statuses of the produced images and different ways of generating (dis)trust in images. The first tier, exemplified by differential gel electrophoresis (DIGE), is characterized by specialized devices processing images as quantitative data. The second tier, exemplified by polyacrylamide gel electrophoresis (PAGE), is described as a routine technique making use of image as qualitative "virtual witnessing". The difference between these two tiers is particularly apparent in the ways images are processed, even though both tiers involve image digitization. Our account thus highlights different views on reproducibility within the two tiers. Comparability of images is insisted upon in the first tier while traceability is expected in the second tier. It is striking that these differences occur not only within the same scientific field, but even within the same family of experimental techniques. In the second tier, digitization entails distrust, whereas it implies a collective sentiment of trust in the first tier.

1. Introduction

1. 1. A crisis of trust in images

The Voinnet affair (around 2015) recounts the story of rising successful biologist Olivier Voinnet forced to retract a hefty number of his publications in retrospect, upon allegations of scientific misconduct concerning supposedly fraudulent or questionable RNA gel blot images (see Figure 1 for an example). The phrasing "Conducted properly, published incorrectly" wraps up the report on allegations of fraud from one of Voinnet's institutions (ETH Zurich, 2015). It summarizes an attitude of trust towards the conducted research but the acknowledgment that something is not quite right in the publication of the results in the form of images (for an account of the repercussions of the Voinnet affair, see for example Guasparre & Didier, 2020).

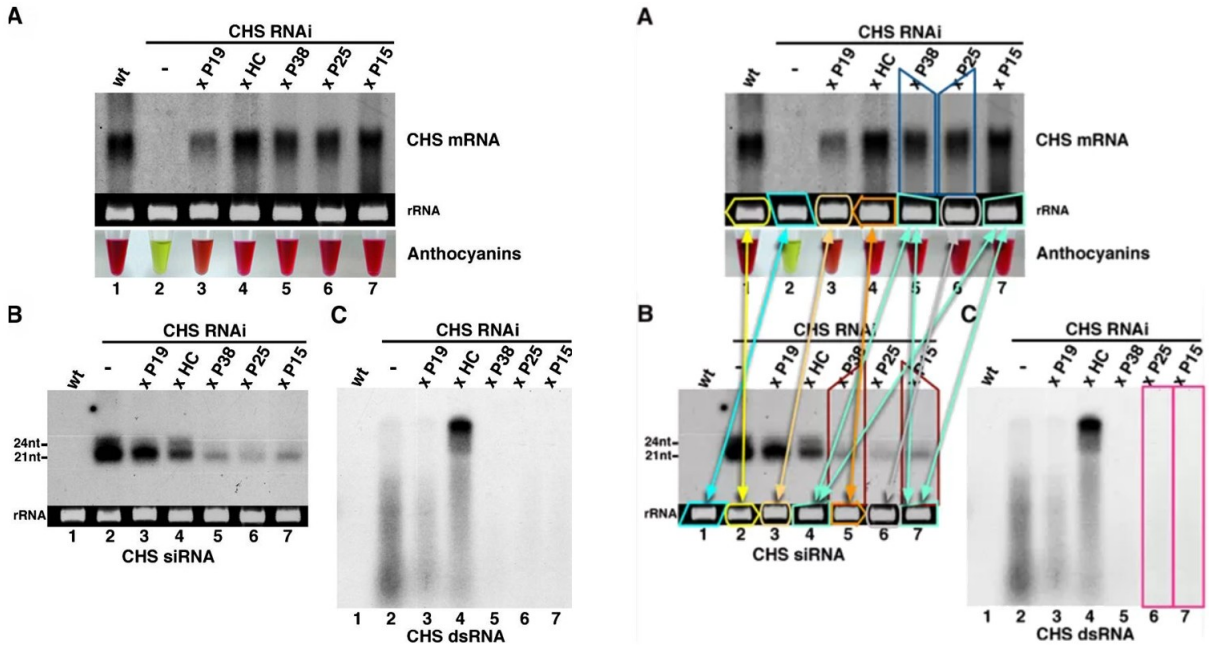


Figure 1 – Left: gel electrophoresis images (RNA gel blot analysis) published in the original paper by the Voinnet laboratory (Dunoyer et al., 2004). Right: an annotated version which highlights suspected fraud involving image manipulations (shuffling, duplication and inversion), posted on PubPeer (2015). The colored arrows and frames demonstrate the presence of perfectly identical image fragments, thereby indicating that certain bands on the gel have been isolated, processed and reinserted in other locations.

What is striking in molecular life sciences is that this affair is not isolated. Numerous similar cases, such as the recent high-profile Alzheimer case (Piller, 2022), indicate a widespread shockwave throughout the whole field. This observation has been the original incentive which

led us to investigate the status of images in gel electrophoresis techniques. Furthermore, the Voinnet affair is particularly revealing because of its numerous legal twists and turns which have emphasized the tensions surrounding the status of images as fabricated (or not) in electrophoresis techniques. At the heart of these contested epistemic roles lies the question of what makes an image worthy of testifying if a piece of investigation could have been conducted properly (or not) yet published incorrectly (or not).

From the vantage point of scientific journals, editors have been concerned with what they perceived as routine and "innocent" yet inappropriate manipulation of digital images. They have introduced guidelines for authors around the years 2000s. According to Frow (2012), three general factors are correlated with this "crisis of trust" in scientific images, especially in the biosciences: the increasing availability of (more- or less-validated) image-processing software (e.g. Photoshop), the increasing reports of misconducts related to image manipulation, and the switch by most journal publishers to electronic workflows. This moment of crisis is thus situated in time: the 2000s. Beyond this narrative of affairs and crisis about images manipulations, we argue that profound transformations of gel electrophoresis as a family of techniques have been occurring in a larger timespan and these transformations do have implications on the way images have been produced, and on the way they are received.

To be clear, we do not deny the importance of scientific fraud as a relevant topic, but the aim of this paper is not to assess scientific practices on moral grounds. Beyond the contentious perception of "affairs" and "questionable research practices", we believe it is fruitful to depict the interplay between, on the one hand material and epistemic transformations and, on the other hand, a crisis of trust about images in the practices of a scientific milieu. This study does not constitute an analysis of the Voinnet affair as such, but an investigation of the roots of an underlying general feeling of distrust following the digitization of images and workflows in life sciences. Yet, these affairs are useful to highlight tensions in the first place.

1. 2. Gel electrophoresis in molecular life sciences

We are thus interested in the evolving status of images produced by electrophoresis as an evolving and diversifying family of techniques. The importance of techniques for separating biological macromolecules like ultracentrifugation and electrophoresis for the adoption of a molecular vision of biology has been stressed in the historiography (Morange, 2020). From its

inital design by Tiselius in the 1930s to its first diffusion in various biology labs in the 1950s and 1960s, electrophoresis has mainly been used "as an analytical method for characterizing macromolecules—in particular proteins, but also nucleic acids [...]" and "[...] as a preparative technique in the separation of both proteins and their fragments" (Morange, 2020, p. 93) thanks to the design of commercial electrophoresis machines (Kay, 1988). With the advent of genetic engineering during the 1970s, electrophoresis became an even more widespread technique used to separate DNA fragments in fledgling sequencing methods. This technique (among others) thus accompanied the development of molecular biology during its two main historical phases (Rheinberger, 2009; Morange, 2020).

It had of course evolved all along these periods by being improved, miniaturized, and "[...] gradually simplified enough to be employed in all biology laboratories" (Morange, 2020, p. 97). This evolution coincided with a diversification of electrophoresis into a family of diverse techniques depending on the nature of studied molecules (proteins, DNA, RNA), the migration medium used (liquid phase, different types of gel, paper), and the way molecules are labeled in order to be detectable. This diversification process thus relied on instrumental development and the advent of a market for instruments makers, particularly with the diffusion of recombinant DNA technologies and biotechnology throughout the life sciences. It has been argued "[…] that these latter events triggered the dissolution of molecular biology into an arsenal of technologies that today pervade all life sciences and a broad range of biotechnological production processes" (de Chadarevian & Rheinberger, 2009, p.4). Among such pervading technologies in "molecular life sciences", gel electrophoresis is one of the most important. Over the last few decades, it has thus become an ubiquitous method, producing a diversity of images from many variations of these techniques.

1. 3. Epistemic status of images

We therefore focus in this paper on one of these instrumental developments by discussing the emergence of quantification methods in protein gel electrophoresis from the 1980s to the 2000s. Our aim is to show how this led to a new variety of ways to produce electrophoresis images. This leads us to describe a two-tiered electrophoresis set of techniques in order to contrast two somewhat ideal-typical ways of producing images associated with two distinct epistemic roles of such images. One tier is concerned with high-end quantitative analysis of results in exploratory research. The other is described as a low-end qualitative routine (and undervalued) confirmation

technique. Beyond a difference in standards, what is striking is the difference in the epistemic statuses of images in the two tiers and the fact that reproducibility as a concept is not even understood the same way. This comparison then offers a framework for understanding contrasted practices regarding imaging in electrophoresis and how they have been differently impacted by digitization. They also have been differently impacted by “reproducibility crisis” rhetorics. We argue that these differences participate in the tensions at the root of the aforementioned scandals.

In other words, we posit that a history of gel electrophoresis on a relatively large timespan helps to understand how and why such a crisis of trust in images appeared in the first place, and how and why at this moment in time, beyond a simplistic view of a "digital turn" [1]. We thus argue that within electrophoresis as a family of techniques, trust in images is impacted by digitization differently. Issues of reproducibility are also not construed the same way. Our description of these issues points out the situatedness of reproducibility, against an overarching normative discourse typical of “reproducibility crisis” rhetorics (Leonelli, 2018).

1. 4. Outline and methodology

In the second part of this paper, we recount a brief history of gel electrophoresis from the moment it became an ubiquitous technique in molecular life sciences during the 1980s to the decline of difference gel electrophoresis (or DIGE) in the 2010s. Our objective is to recall summarily how electrophoresis works, then to give an account of relatively recent developments with respect to previous historiography, and finally to highlight the emergence of two ideal-typical tiers in times of quantification and digitization. In the third part, we then explore the epistemic and material transformations of images at the turn of the 2000s in each of our two tiers and their consequences on reproducibility issues. On the one hand, we make use of the notion of "collective disciplining" (Cambrosio & Keating, 2000) to depict how quantification and automation strive in the first tier. On the other, we describe how a general feeling of distrust about digitized images emerges in the second tier. We then surmise that reproducibility issues are envisionned in orthogonal ways in both tiers, as comparability is the virtue of choice in the first tier whereas traceability is the one in the second tier. Finally, we conclude by turning back to the significance of scandals like the Voinnet affair in the framing of our descripition.

[1] The “digital turn” we are talking about here is about the production of images. A simplistic view of that turn would be to consider that the digitization of this production is a simple process associated with a linear, inevitable and one-size-fits-all progress.

Our analysis, in the second part, of the technical and diachronic transformations of gel electrophoresis is based on a set of interviews (five sessions with seven interviewees) coupled with published primary sources. These interviews were conducted using a semi-structured method (average duration of each session: one hour). The framework used to conduct the interviews consisted of questions aimed at exploring various themes related to laboratory practices for gel production and image processing, the evolution of editorial requirements and their impact, the trustworthiness of gel images in the literature. In addition, these interviews offered an opportunity to collect gel electrophoresis images from various stages of the interviewees' careers.

The seven participants work at Belgian universities or corporations. They belong to different professional categories (PI, junior researcher, technician) and work in various sectors (academic or industrial) in different laboratories (biomolecular physical chemistry, protein engineering, cell biology, biopharmaceutical R&D). In this paper, when quoted, the profile of each interviewee will be briefly described according to three factors: work experience (young scientists vs experienced scientists), area of activity (academic sector vs industrial sector) and job function (technician, researcher, professor, etc.).

Our historical description is of course strongly influenced by the participants' profile and career, and leans more towards proteomics. We nonetheless consider that the highlighted innovations entail epistemic consequences (discussed in the third section of the article) that contribute to the current crisis of trust in electrophoretic images in general.

2. A brief history of gel electrophoresis (1970s-2010s)

2. 1. Gel electrophoresis: an overview

Gel electrophoresis is a method used to analyze (or separate the components of) a complex mixture of biomolecules (typically proteins, nucleic acids or their fragments). The separation of macromolecules is based on the migration of these charged biomolecules in an applied electric field, through a hydrated gel network as stabilizing medium. Nowadays, gel electrophoresis encompasses a wide variety of experimental techniques that vary according to the gel format (horizontal or vertical slab gel, tubes, capillaries, etc.), the gel type (starch, agarose gel, acrylamide gel, etc.), the labelling (organic dyes, silver staining, fluorescent staining,

radioactivity, etc.) or the post-electrophoretic identification of molecules (blotting, mass spectrometry, etc.).

From the late 1990s to the 2010s, gel electrophoresis has been characterized by the coexistence, within the aforementioned variety, of two techniques that we single out as exemplar all along this paper, as shown in Figure 2 timeline. Figure 3 shows a general comparison between these two techniques. The "canonical" gel electrophoresis experiment established in the 1950s and known by generations of undergraduate biochemistry students can be exemplified by polyacrylamide gel electrophoresis (1D PAGE), with no major evolution since the 1970s. It is widely used in all molecular life sciences laboratories and provides quick qualitative analysis of samples. Using pipettes, samples and molecular weight size markers are loaded onto the top of the gel surface into adjacent wells. The biomolecules migrate downwards under the influence of the electric field, causing separation of the various components based on their molecular weight differences. After migration is complete the biomolecules can be visualized through gel staining, which involves binding a colored molecule to the components. The resulting colored biomolecules can then be compared to a control sample to determine their presence or absence. Molecular weight size markers are used as a calibration tool to estimate the size of each detected biomolecule in the sample. A photograph of the gel is taken and the gel itself is dried and stored, typically in a lab book (see the left part of Figure 3).

The second technique is differential gel electrophoresis (DIGE). Since the 1990s, it has gained traction to assess, quantitatively and comparatively, proteins abundance in analyzed samples. This technique involves optical fluorescence detection of differentially labeled proteins that are separated on the same gel through two dimensional gel electrophoresis (2D PAGE). In order to distinguish the two analyzed samples, each is pre-labeled with a different fluorescent dye (Cy3 or Cy5). An internal standard is prepared by mixing the two samples with a third fluorescent dye (Cy2) and serves as a reference for quantitatively comparing the detected constituents. The three samples are run within the same gel; components are separated according to their isoelectric point in the first dimension (the x-axis) and according to their molecular mass in the second dimension (the y-axis). The gel is scanned at the excitation wavelength of each dye one after the other. Computer-aided image analysis is performed in order to detect spots and evaluate intensities and spot volumes. Measured intensities of Cy3 and Cy5 are normalized versus Cy2

intensity. By running multiple replicate samples, statistically meaningful differences in protein abundance can be highlighted between tested conditions (see the right part of Figure 3).

The visual description of this two-tiered technique (Figures 2 and 3) of course doesn't do justice to the diversity of gel electrophoresis. Yet, it allows us to highlight the particular material context in which the crisis of trust in scientific images takes place. We will now describe how the emergence of the two tiers occurred.

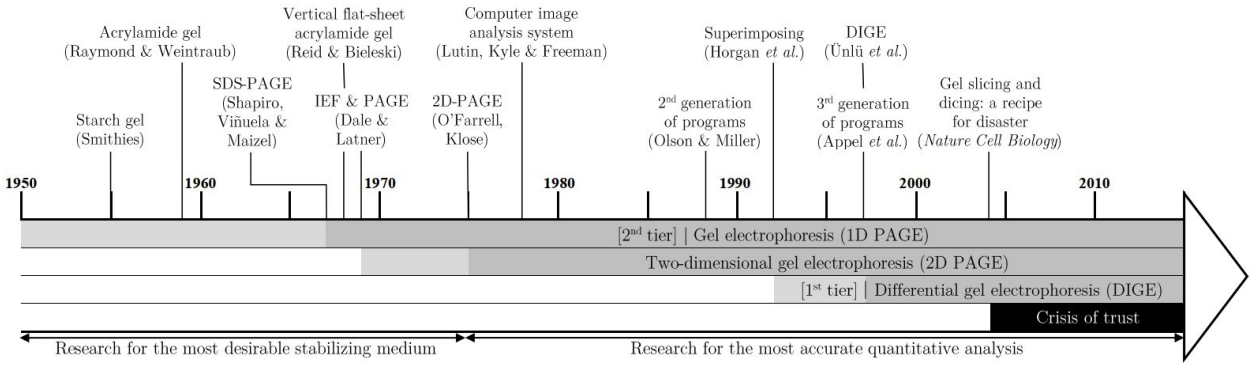
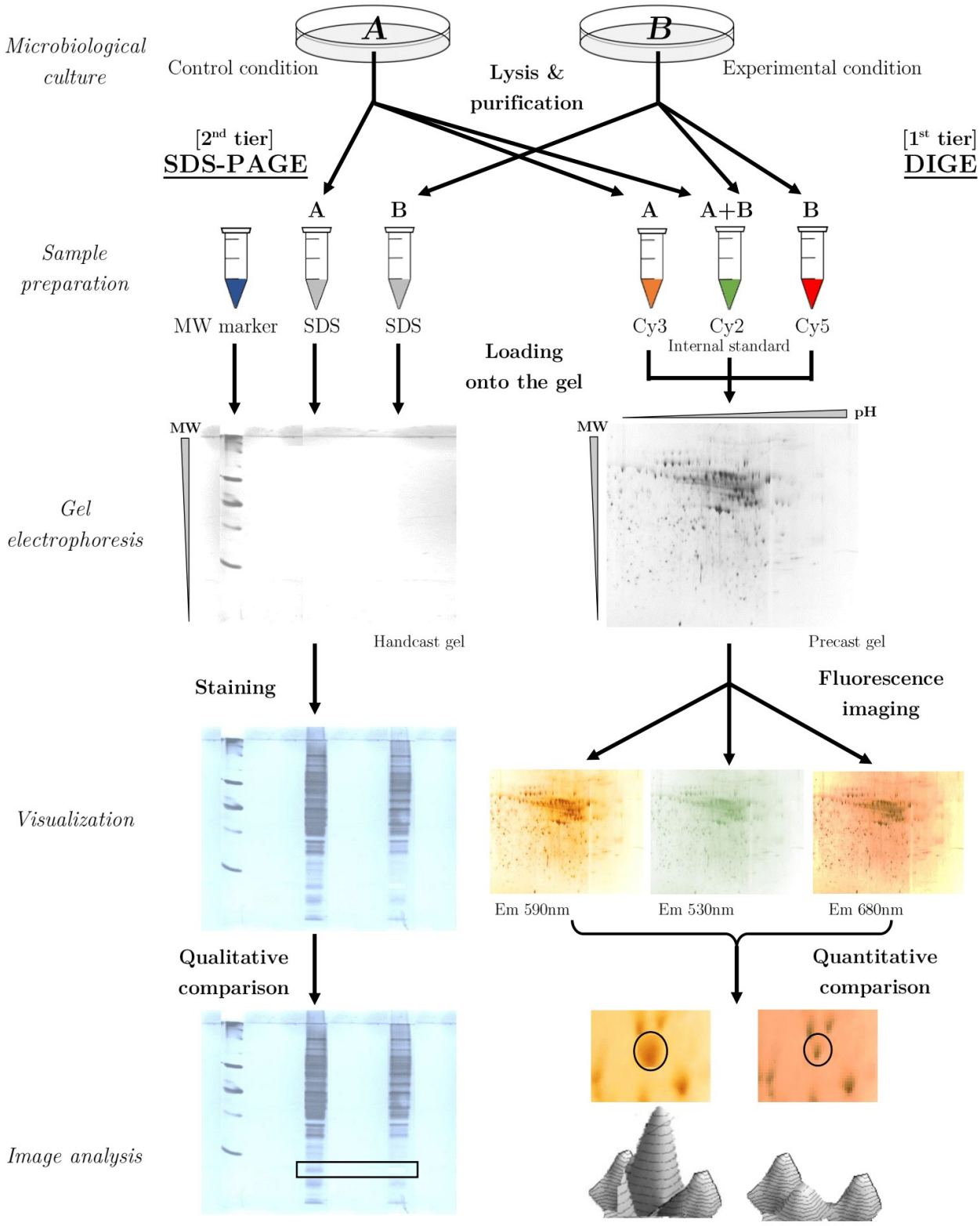


Figure 2 - Chronological evolution of innovations within gel electrophoresis between the 1950s and the 2010s. Prior to the 70s, innovations were focusing on medium optimization for the separation of biomolecules. From 2D-PAGE, the focus shifted towards quantitative analysis of gels. This article delves into this period characterized by the coexistence of the two tiers and the emergence of a crisis of confidence in images within life sciences. CC BY-SA Callaerts, Hocquet, Wieber.



- Visual band detection.
- Binary analysis (protein is/is not detected).
- No replicates.

- Computational spot detection.
- Calculation of abundance from normalized spot volume (Cy3/Cy2 and Cy5/Cy2) ratios.
- Statistical analysis of replicates.

Figure 3 - General comparison between 1D PAGE (2nd tier), left column, and DIGE (1st tier), right column. MW (Molecular Weight); SDS (Sodium Dodecyl Sulfate); Em (Emission maxima of dyes). CC BY-SA Callaerts, Hocquet, Wieber.

2. 2. Precast gels as industrial standard

The first decisive shift in the evolution of electrophoresis methods from the original "moving boundary" technique to gel electrophoresis coalesced from the effort of scientists (from physical chemistry to immunology) as they looked for the most desirable conditions to optimize the migration of samples and separate biomolecules efficiently (Chiang, 2009). This research paradigm reached its peak during the 1960s with the development of key innovations. Polyacrylamide enabled the formation of stable, flexible, and transparent gels that could be easily dried to preserve the original separation pattern (Raymond & Weintraub, 1959). Sodium dodecyl sulfate (SDS) was introduced as a denaturing agent for rapid and simple estimation of protein molecular weight and subunits (Shapiro, Vinuela & Maizel, 1967). The use of thin slab gels (as opposed to tubes in disk electrophoresis) improved electrical simplicity and safety while also expanding the range of possible post-electrophoretic applications, such as autoradiography (Reid & Bieleski, 1968).

The improvement and diversification of gels, a user-driven innovation until the 1970s, and the promise of a booming “genetic engineering” market led industrial actors to design useful, time saving and standardization enhancing so-called “precast gels”, transforming a fastidious experimental setup into a routine technique. As a matter of fact, self-prepared gels require a specialized expertise of a trained laboratory technician (in order to avoid leaks, to store homemade solutions, to monitor polymerization speed, to prevent gel dehydration, etc.). As one experienced researcher in academic sector states “it required much dexterity to create these gels, [...] for the gel to last, like a piece of art, which was a goal [...] in itself”. By contrast, new commercially obtained gels are ready to use, offer greater convenience and provide an extended shelf life compared with handcast gels. In one experienced lab researcher’s words, “the commercialization of precast gels, [...] has really helped to reduce the variability of the electrophoretic result”. The aforementioned researcher insists on more accuracy and more reproducibility: “It often happened that an expert made a gel, and then another person wanted to make the same gel but obtained different results. This isn’t acceptable from a scientific point of view. [...] But this is no longer the case [today]".

Visualization has also been taken over by these industrial standards: patented dyes, markers and buffers were designed in order to save time, decrease toxicity and increase sensibility. Commercial kits and associated bench-top instruments (ensuring electrophoretic migration and visualization) became ubiquitous, diverse and standardized. Gel electrophoresis was no longer necessarily confined to personalized protocols using homemade solutions. Commercial kits diminished local errors for each step of the protocol through standardization. These commercial kits involving precast gels offered ready-made commercial buffers, migration in a more controlled environment (homogeneous electrical field, fixed temperature, etc.) and higher purity dyes for staining. The combination of manufactured devices with dedicated precast gel and staining kit provided thenceforth an integrated system that involved minimal manual involvement for the ones able to afford them[2]. Roughly speaking, these industrial innovations of the 1980s are the common setup of what has since become a routine bench practice, one that has only marginally changed afterwards to this day.

The end-product of such apparatuses was once a stained gel which had to be dried, preserved and stored. An analog camera was used in order to draw attention to some selected gels. Imaging was perceived as a supplementary step of the process of electrophoresis. Using techniques of manual and instrumental enhancement, scientists tinkered with the conditions that improve the visibility of certain materials. Indeed, the recording technique of experimental results was plain photography until the 1990s (from photographic film, instant cameras to digital cameras, sometimes coupled with transillumination). The quality of images shared as outputs was dependent on the optimization of non-specialized photographic devices and comparability was not even pursued. Gel images were made on photographic films and stored in lab notebooks[3]. The epistemic function of gel electrophoresis images is in this case to witness the appearance of stains (as illustrated in the left part of Figure 3) as a confirmation of the presence of expected biomolecules. When such images are published, they become a "virtual witness" whose purpose is to convince, within a framework that Shapin & Schaffer (1985) describe as a "litterary

[2] Precast gels did not immediately and universally supersede handcast gels. In this section, we describe precast gels as the first technical innovation that paved the way for the emergence of what we call the “first tier” of gel electrophoresis. Home-made gels are still regularly used in many laboratories today, as they remain relevant and useful in certain fields of application which refer to the “second tier”.

[3] "There was indeed [...] a tripod camera. We had bought a macro lens to take pictures, with obvious comparison difficulties due to the view angle, the distance, etc. All of this [the results] was quite difficult to exploit. [...] I think that each laboratory had its own means, its own camera, took pictures and stuck a photo on a poster in the case of a conference for instance". [Experienced researcher in the academic sector]
"We took pictures with a Polaroid. Before 2000, it was very basic. This allowed us to keep the image of the gel but without being able to perform in-depth analyzes". [Experienced researcher the industrial sector]

technology". As a matter of fact, Amann & Knorr Cetina (1988) insist, while describing autoradiographic gel electrophoresis, that the production of publishable images requires a time-consuming and laborious process of selection and transformation of output images, to transform "data into visual evidence".

2. 3. Gel docs as incorporation of image production

The late 1970s marked the second decisive shift in the evolution of electrophoresis methods. The theories involved in electrophoresis itself were well understood. Even though densitometric analyses had been undertaken within the process of finding a stabilized medium since the 1950s, quantification issues became specifically studied in the 1970s (Zak, Baginski & Epstein, 1978). The development of 2D PAGE had increased the amount of potentially available information that is observable in complex biological samples (O'Farrell, 1975 and Klose, 1975). A shift in molecular markers (as well as the associated laboratory equipment) occurred to reach more direct and sensitive detection methods (Schlötterer, 2005). This new emphasis on quantification and detection methods has led to a related move towards the use of molecular data and automation, which includes both the technological and procedural aspects of gel analysis as well as the automation of inferences and decision-making through software and hardware.

Gel electrophoresis became an area of interest studied by scientists from various fields, with accurate quantitative analysis in mind. More specifically, this era is characterized by the growing influence of an emerging scientific community dealing with information and communication technologies in biosciences (Dowsey, Dunn & Yang, 2003). Collaborative practices between biochemists and a fledgling biomedical computing scientists community led to innovations published in newly founded journals (e.g. *Computers and Biomedical Research*, *Computers in Biology and Medicine*, *International Journal of Medical Informatics*, etc.). These innovations focused on software tools for quantitative analysis of (mainly two dimensional) gels (Lemkin et al., 1979).

These tools were leveraged in the form of a new specialized device, the so-called "gel doc", a device that was (and still is) integrating the whole experimental setup into an apparatus taking charge of image recording (thanks to scanning with a digitizing camera or laser densitometer), image processing, image analysis, image handling and finally image presentation. Computer image analysis systems for 2D gels were first described by Lutin, Kyle & Freeman (1978).

During the following years, several other similar systems were developed, including the first commercial ones. Computer image analysis systems were perceived as an opportunity for standardization within quantitative gel electrophoresis. In one experienced researcher's words, "it [gel doc] was actually a system within a workstation; […] a box wherein there was a dedicated camera linked to a piece of software. Then, standardization began: pictures were always shot at the same height, brightness was always equivalent, … […]. It [the device] had the advantage that we could make use of the gel and we could start making area [...] or intensity calculation. […] Software made it possible to match gels and to correct any offsetting". Standardization led to comparability, which became the main purpose of quantitative image analysis investigations, in contrast with previous habits[4]. At this stage, images in gel electrophoresis turned from analog to digital. Two major devices for image acquisition in gel electrophoresis emerged: scanners and charge-coupled device (CCD) cameras. In both scanners and modern cameras, the recording device turned into a CCD chip; the output was therefore an electronic signal that could be digitally interpreted, instead of plotted on a paper graph.

However, powerful image analysis systems remained relatively rare because of problems in the design, pricing and software of available systems in the 1980s and early 1990s. The first generation of software, without a graphical user interface, required a steep learning curve and technical experts. Programs using graphical user interfaces on computer workstations appeared in the late 1980s. However, such pieces of software needed exceptionally equipped workstations. In addition, data was recorded in gel docs within a proprietary computer file format, one that was specific to the manufacturer: "For the gels, […] the format was initially device-specific. […] You know, at that time, there was [for example] the Amersham company; you had to use the Amersham device, with the Amersham [analysis] system" [Experienced researcher in the academic sector]. Images of gels were not easily shareable among different laboratories[5]. As a result, costly image analyzers offered too little performance at too high a price (Ramm, 1994).

[4] "You had to take an image […] and then spend hours with your tiny eyes trying to spot [a difference] between condition A and condition B. Were they different? Yes-No. Were they really in the same area? On a [two-dimensional] gel, you could have hundreds of spots. […] It's a contentious topic: there is an obvious possible bias , obvious subjectivity […] I think that digitization has reduced this subjectivity when you are interpreting results from your gel". [Experienced professor in the academic sector]

[5] "When the manufacturers market new devices, we can see that they now mind the capacity to produce data that can be used by other software. That wasn't the case before: they had their own software and they were like "you're on your own, we are selling the software, nothing more". […] This is a big deal if you deal alone with this software and you're not a mathematician. It was originally command lines". [Experienced researcher in the academic sector]

During the 1990s, advanced image analyzing systems became available to many laboratories. Image capture technologies had shown great progress thanks to CCD cameras (Miura, 2001). Software has been redesigned as an affordable and user-friendlier image analysis system for personal computers. Thus, modern gel docs have been incorporating powerful and cost-effective hardware, new generation software, and high-performance devices for image acquisition. New features in second-generation gel analysis software packages have made accurate comparison of two-dimensional gel images possible (Olson & Miller, 1988). In the late 1990s, a new two-dimensional approach called DIGE was introduced by Ünlü et al. (1997) by combining superimposing of gel images (Horgan et al., 1992) with detection of (newly patented) cyanine based fluorescent protein labels.

One of the main purposes for development of DIGE was to overcome the poor repeatability of 2D PAGE, namely the inherent technical and systematic variability that affect the generated patterns and spots intensities on gels. DIGE involves running differently labeled samples in a single gel; a concept called “multiplexing”. This method is accomplished by tagging the two samples with two different dyes, running them on the same gel, and then "post-run" imaging of the gel into two images and finally superimposing these two images. An internal standard with a third dye is used in order to calculate normalized intensities and performing rigorous comparison between images. Multiplexing has led to the ability to design experiments that vastly reduce gel-to-gel variation, resulting in biological replicates being used for statistical analysis, with the ability to detect very small changes in relative protein abundance[6]. This highly reproducible and sensitive form of gel electrophoresis paved the way for quantitative research and served as a prominent driving force to spread gel doc apparatuses in the community, thus constituting a profitable business for industrial actors. In contrast to more "qualitative" gel electrophoresis users, 2D PAGE and DIGE experts were aiming for increasing standardization, through more and more sensitive dyes, high-resolution CCD chips, controlled image acquisition, powerful data analysis and extensive file storage. The third-generation of two-dimensional gel image analysis software is therefore characterized by the introduction of algorithms derived from the development of artificial intelligence and machine learning (Appel et al., 1997).

[6] “To try to standardize this approach [comparison between 2D gels], there is what is called 2D-DIGE. […] [This is] a revolution in the early 2000s that consisted of labeling [two different] samples […] one with a red fluorochrome, the other with a green fluorochrome. The protein samples were mixed [and] you added an internal standard labeled with another color to normalize the fluorescence intensity of the gels. So now, you can afford to make (biological, technical, etc.) replicas with several gels. Digitization and quantification hugely contributed to a much cleaner and more objective detection of different spots [between 2D gels]”. [Experienced professor in the academic sector]

At this stage, image production was incorporated within gel electrophoresis data processing. Preparation of an internal standard and gel imaging (namely, image production, analysis, modification and comparison) were included within the DIGE protocol (Beckett, 2012). In this case, the end-product is neither a dried gel nor a digital image of a gel: it actually is a set of various densitometric pieces of data, in a shareable format, generally communicated through histograms, exemplifying averages and standard deviations. Gel electrophoresis image then becomes a piece of quantitative data, something internal to the instrument, which is being processed in the context of a new biomolecular analysis method.

2. 4. Tinkering vs industrial standardization

We have thus described the historical context in which arose what we called a “two-tiered gel electrophoresis”. The first one, DIGE, embodies the result of two decades of research focused on accurate quantitative analyses, thanks to gel docs as industrial standardized apparatuses. The second one, 1D PAGE, a routine confirmation technique, provides quick qualitative analysis. In both cases, imaging, through various optical devices using CCD as main core technology, became then an additional systematic step to gel electrophoresis data processing. Digitized image is therefore the predominant data type used in both qualitative and quantitative gel electrophoresis. Consequently, the preservation of dried gels gradually evolved into digital storage of gel images[7]. Yet, the main distinction between these two tiers consists in the system used in order to perform imaging and the subsequent status of gel electrophoresis images. Regarding instrumentation, 1D PAGE commonly involves a digital camera or a document scanner system for office use whereas DIGE requires a gel doc apparatus. Regarding images, the outcome of 1D PAGE experiments remains a representation of a gel. Unlike DIGE, imaging is not incorporated within the instrumental device. Imaging often involves non-specialized optical devices coupled with more or less specialized image processing software, such as the open-source ImageJ, designed by scientists, or the mainstream, proprietary, and multi-purpose Photoshop. A kind of "expert tinkering" is then the main way to perform "qualitative" gel electrophoresis, resulting in digital images that are handcrafted on the fly. As a matter of fact, montage is an essential part of the process of transforming laboratory data into images that

[7] “Previously, […] there was a whole process of drying gels which people kept in folders […] That was before digitization, […] people kept them in their lab notebook”. [Experienced researcher in the academic sector]
“In the late 1990s, scanner for office use emerged, so the drying of gels was slowly abandoned: either we took a picture or we scanned the gel”. [Experienced technician in the academic sector]

support a specific argument within a scientific publication. Qualitative gel images are barely comparable but easily shareable and indeed easy to manipulate, for better or for worse.

In contrast, DIGE apparatuses offer an industrial standardization of the way images are being produced and processed. However, gel docs have faced usability and interoperability issues. Until the 2000s, gel docs were proprietary user-unfriendly black boxes; the development of various devices by various manufacturers led to different standards, regarding file formats for example. Since then, gel docs have been diversifying; some manufacturers market imaging systems encompassing cutting-edge technologies which require high-level expertise to be used, while others seek a wider user base by including numerous automated technical processes. In this second case, gel docs have been designed to be as modular as possible to encompass a growing variety of features. With the adoption of the TIFF format as a shared standard, densitometric data obtained as end-product of quantitative analysis became more comparable and shareable, offering some level of interoperability[8].

2. 5. Aftermath: (re)constructing images

Finally, a golden age of DIGE spans from the early 2000s to the mid-2010s. Although DIGE is typical of what we call tier 1 electrophoresis because it allowed efficient and reproducible quantification, liquid chromatography-mass spectrometry (LC-MS) has since taken over as a cutting edge tech. Despite the fact that DIGE is still presented as a complementary technology in 2015 (Arentz et al., 2015), it is identified as obsolete by interviewed scientists nowadays[9]. Still, gel doc as an apparatus remains the main tool that ensures data acquisition, processing and storage, in more recent technologies. Therefore, images are still fully incorporated within an electrophoretic protocol. Yet, this does not mean that the "virtual witnessing" function of electrophoresis images has completely disappeared. "Qualitative" gel electrophoresis (and potential underlying tinkering) survived as routine and ubiquitous experimental analysis. Sharing

[8] “Initially, the [image] format was specific to the device. So we couldn’t do anything except working with the [marketed] system. Then, exports in TIFF emerged, TIFF could be used in other software packages like the open-source ImageJ [...] which made possible to further analyze the [images of] gels trough different pieces of software”. [Experienced researcher in the academic sector]

[9] “Mass spectrometers have gotten better and more accurate, and we realized that in the same small spot, we would find several different proteins. So it was crucial, because it highlighted that in fact we couldn’t tell if the evaluated intensity was due to the protein x, y or z because they all had the same location. So this beautiful technique [DIGE], which had evolved so well, was doomed. [Experienced professor in the academic sector]

“Mass spectrometry has - let’s say - killed a lot of techniques. […] IEF, 2D gels, etc. […] amino acid analyzers as well. […] 2D DIGE […] became obsolete few years ago. It was very trendy from the late 90 to 2010-2015. Now it’s completely outdated”. [Experienced technician in the academic sector]

selected digital images of gels is still the main outcome of this method, even if it is not always published anymore.

It is also interesting that, even for some quantitative methods that do not initially involve image within their process, digital gel images are being numerically reconstructed, as in modern gel docs involved in capillary electrophoresis. Electropherograms of several biological samples can be gathered within a single reconstructed gel, allowing an overview of several experiments on a single image.

This new practice of image reconstruction sheds light on the dual function of (published) gel electrophoresis images. On the one hand, they can function as qualitative "virtual witnessing" of what has been "observed" on a given gel. On the other, they can be used as a convenient yet artificial representation of quantitative gel electrophoresis results, as a mean to showcase human-readable outputs. According to scientists in the field, images are preferred to communicate electrophoretic analysis results (even when it is optional, in DIGE, or totally artificial, in capillary electrophoresis) because this kind of data is perceived as an integral part of the gel electrophoresis identity. One experienced technician in the industrial sector recounts how he handles the presentation of results: "In front of the capillary, there is a kind of sensor which detects things and an artificial image can be reconstructed. We can obtain lanes as if we had a [real] gel, […] but actually, there are simply molecules in front of a sensor, so it produces peaks of [detected] molecules. Personally, […] I always showcase the reconstituted gel because it is much more meaningful for people. […] It will depend on the individual, but for a person who has been doing this for a long time, it will be much more revealing […]".

Since the emergence of DIGE and gel doc development in the late 1990s, images are embodying an ambiguous opposition between two epistemic roles, more or less salient depending on the incorporation of image production within the gel electrophoresis data processing. Figure 4 provides an overview of the equipment used by the interviewed scientists to generate gel images with different functions, for three distinct electrophoretic analyses.

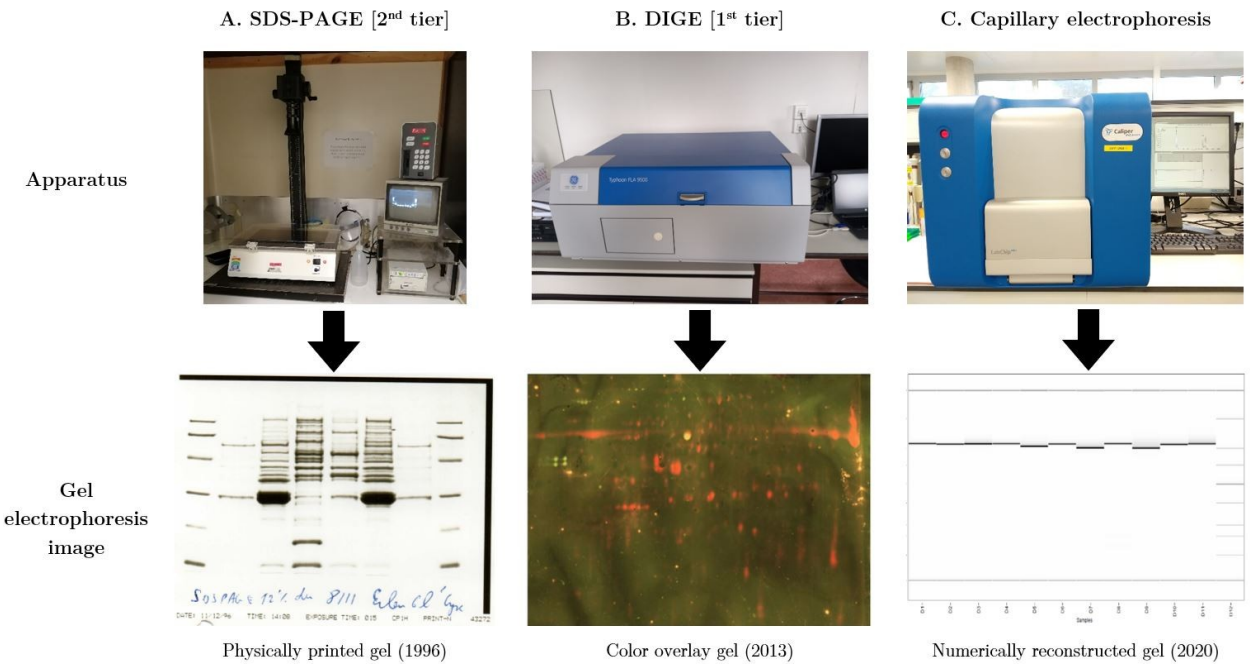


Figure 4 - Apparatus and associated gel electrophoresis image for SDS-PAGE (2nd tier), DIGE (1st tier) and capillary electrophoresis. Column A shows a video graphic printing system (including a dedicated camera, a benchtop transilluminator, a monitoring screen, and an analog monochrome thermal printer) and the resulting printed gel, which was generated in 1996. Column B shows a laser scanner (providing several imaging modes) with a monitoring screen and the resulting digital image of a color overlay gel, which was generated in 2013. Column C shows a fragment analyzer and the resulting gel, which was numerically reconstructed from capillary electrophoresis of several samples in 2020. CC BY-SA Callaerts, Hocquet, Wieber.

3. Epistemic consequences: reproducibility in a two-tiered electrophoresis

3. 1. Image digitization: a variety of processes

The 2000s have been characterized by Frow (2012) as a shift in publishing practices in biomedical sciences, especially regarding the publication of digital scientific images. Images of gel electrophoresis in particular came in the spotlight because of a perceived crisis of trust. According to Frow, the digitization of manuscript submission, combined with an increased resort to image beautification entailed a change in author guidelines. Image modifications that were tolerated or not even noticed in "analog photos" were becoming frowned upon. Our analysis, in parallel to Frow's, is based on scientific practitioners interviews. They are not journals editors but the producers of scientific images at the bench for eventual submission to a journal.

According to them, publication guidelines regarding image production turned from leniency to stringency[10].

Gel drying or photography pinning in a lab-book were once the storage of choice for eventual "virtual witness" use. Once digital image storage had taken over, image digitization has become the pivotal issue in both tiers. A digital image of a gel may come from a variety of processes. It might be a digital image produced by a gel doc comprising image production and processing thanks to a dedicated patented piece of software. Or, it might be shot by a high end CCD camera, or a plain digital camera, and then processed by a piece of scientific software, or processed by Photoshop, including preliminary gel drying or not. The two tiers we have defined as ideal types simplify an array of diverse practices. Yet, they are useful to understand what is at stake on both sides of this continuum. What is new and noteworthy in a "digital turn" during the 2000s is the two different ways image processing is conducted in each of these two tiers even though both involve digitization.

Differences between gel docs image processing (first tier) and "tinker" image processing at the bench using lay tools (second tier) can be understood in the context of exploratory, high-end, quantitative experiments vs routine, cheap, and fast confirmation techniques. In both cases, the migration in the gel is the same, but image production, processing and storage differ, even though they both include digitization. On the one hand stands an ambitious and exploratory technique, on the other strives a routine, mundane and even disregarded scientific activity. The software processing step of image treatment reflects this divide. Bluntly put, in house, commercial industrial software is contrasted with plain scanner and Photoshop. The industrial alternative of the first tier greatly enhances comparability, even across research groups, albeit within a commercial ecosystem; on the other hand, in the second tier, the homemade solution uses a piece of software with a bad reputation for data integrity, because of its mainstream use as a beautifying tool.

[10] "The moment it becomes standard, the moment it becomes popular, they start to put guidelines. Proteomics – the field I'm working in – had very weak guidelines, almost non-existent. Now, they impose huge constraints, because everyone working [in this field] are using it as a standard [imagery] tool. Now, they have to put relatively strict guidelines to publish data." [Experienced researcher in the academic sector]

3. 2. First tier as collective disciplining

In DIGE, the paragon of the first tier, the gel doc produces and processes an image within the instrument. It is even possible to process and compare different gels. Several images are part of the process and they can be statistically analyzed. Repeatability thus entails a change in presentation of results: instead of a single image, histograms as a concretion of several images, each representing a single experiment, become feasible. Human-readable images may be digitally reconstructed as a statistical average of repeated experiments for easier cognitive reading by a human (because of its traditional presentation format) but the evidence lies in a quantitative treatment of several collated images.

This way of producing evidence is a consequence of the growth of a collectively shared technical expertise. The outbreak of gel docs corresponds to a fledgling market for industrial scientific instruments introducing more and more automation and computerization to gel electrophoresis, especially regarding data treatment, and is linked to an ambition of quantification. Industrial software tackled post-production and statistical treatment turning images into a less relevant output. This output, as histograms and bar charts, is more concerned with issues of statistical treatment, repeatability and participates to the advent of an industrial standard.

This technical expertise possesses a long history of industrial computerization. Apparatuses devised to integrate the numerical production of images within the automated experimental protocol are handled by computer experts on the instrument production site but they also need computational expertise on the user side. Technical expertise is thus not only the product of an industrial, instrumental research on numerical processes mixing image production and treatment. It comes also “from the formal and informal learning procedures involved in laboratory work […] leading to the stabilisation of practices” of users (Cambrosio & Keating, 2000, p. 251). This is what Cambrosio & Keating (2000) call a “collective disciplining” that “lies in the particular mix of equipment and disciplined performance” (p. 251).

This standardization process is thus twofold in the sense that it involves an industry on the one hand but also a community of users on the other. It is also twofold in the sense that it involves not only data treatment, automation and computerization but it also influences the users bench practices. For example, in DIGE, multiplexing involves experimental procedures before data acquisition. This mix of standardized procedures, automation and quantification corresponds to

“handling the apparatus, analysing the data and representing them” (Cambrosio & Keating, 2000, p. 265) and implies the “collective disciplining” that builds a consensus within the community, and thus trust in images. Within this framing, digitization appears as “naturalized”, and even compatible to an objectivity ideal. Industrial standardization in turn is perceived by actors as enhancing repeatability across laboratories.

3. 3. Distrust in the second tier

In the second tier, homemade gel scanning (plus eventual photoshopping) remains the easiest solution for routine analysis. As experiments become repeatable, the most beautiful, typical gel gets selected because of its exemplary status (it shows better stain separation, the absence of artifacts clarifies the picture, etc...). Repeated experiments are barely compared. Their purpose is to select the most typical picture out of a repetition of the same experimental process. No averaging or statistical treatment of any kind is performed.

Even though some semi-quantitative analyses do exist in some quarters of the very broad spectrum of uses of 1D-PAGE, qualitative analysis remains the use of choice in the second tier. To achieve exemplary status, the image as output of an experiment relies on aesthetic criteria. Rasmussen (1993) has shown in his study of electron microscopy images that aesthetic criteria are epistemically relevant for practitioners, to decide whether images correctly describe the biological phenomena at stake. In our case, they participate to the construction of exemplarity, as “beautiful images” convince because they are supposed to make reference to careful practices.

Amman & Knorr-Cetina (1988), in their description of electrophoretic autoradiographs, put forward the collective work towards the “fixation of visual evidence”. They show that through negotiations among lab participants and alterations of gels like cropping, cutting and glueing lanes together, pointing, etc... each image of a gel that is destined to be presented beyond the laboratory (for publication or conference purposes) is constructed out of these alterations, precisely because it is transforming the output of an experiment into a piece of evidence that has to be made clear to the outside world. Beautification, as for example in the removal of ugly stains that hamper the visualization of what is at stake, is thus part of these practices, that remain largely tacit. These “analog” practices are actually the ones mentioned by Frow as the ones that pose no problem to journal editors while their digital counterparts are frowned upon.

Digitization of such practices thus entails distrust in the sense that beautification practices that were once routine but invisible are now exposed as suspicious. Actually, technical expertise in the second tier suffers from a bad perception of lay tools. Photoshop, in particular, is very easily demonized. For this routine kind of electrophoresis, standardization and accountability are more difficult to achieve because of the high diversity of lab (bench) and software (desktop) practices. In contrast with the first tier, there has never been formal training on image analysis for young researchers and students performing second tier gel electrophoresis. Also, the tools of numerical post-production in gel electrophoresis are very far from a trusted scientific device; instead, it is quite the opposite: Photoshop is not scientific software, its user-base activity is typically about altering (or "beautifying") images. It has a very bad reputation in terms of data integrity (photoshopping as a verb is hardly perceived as a legit activity). Its very user interface is even appropriate to bricolage: its menus are full of tinkering analogies like cropping, pincels, scissoring, blurring... These are the typical vocabulary of retouching (Manovich, 2011). This is also remarkable that the same vocabulary is used in the analog practices of gel alterations we described just above. Finally, its friendly user interface makes Photoshop easy to use to non-experts. This undervalued expertise in this kind of post-production inspires general distrust, as opposed to the situation in tier 2 before digitization. It is of course also opposed to specifically designed scientific software used by well trained, computational savvy scientists in the first tier.

As Porter (1995) has devised, this kind of distrust arises when there is no uniform acceptance of what technical skills and training are needed. In other words, scientists did not manage to reach consensus on a definition of numerical image post-production sound practices and literacy, in contrast to the collective disciplining within the first tier. Thus, reproducibility doesn't have the same status in the two tiers. Unlike collective practices negotiated at the bench described by Amann and Knorr Cetina, beautification in the Photoshop era (mostly solitary, not formally trained, and using lay tools) does not benefit from the same relationship to perception of careful practices. Analog practices that once were consensual and tacitly shared are being replaced with digital practices that unsettle an ideal of objectivity.

3. 4. Comparability and traceability

Technical expertise as a warrant for reproducibility is not regarded with the same level of trust in the first and second tiers. Technical expertise is typically associated with industrial standards and is not questioned in the first tier. Even though these industrial standards could be criticized as

black boxes, they are barely concerned with the tensions of a trust crisis in images. In the latter case, digitization entails distrust, whereas it implies a collective sentiment of trust in the former.

In her study of journals guidelines regarding images in molecular biology, Frow (2012) has addressed how "mechanical objectivity" (Daston & Gallison, 2007) is seen as an ideal-type by journal editors who endorse a naive vision of photography. Numerical post-production of images is thus worrying for these editors whereas "analog" post-production at the bench was not concerning at all. Technical expertise of lab practices were trusted in analog times while suspicion might appear in the digital era. Frow argues that this is typical in a field where "analog representations have long been commonplace, such as cell and molecular biology" unlike other scientific fields "where digital imaging has been used from the outset" (Frow, 2014, p. 254).

Finally, the epistemic status of images in both tiers is not only different in their production, it is also different in the context of their use within a publication. Images serve very different purposes, and reproducibility concerns are very different indeed. In the first tier, reproducibility is very important for the image production and processing itself. The image, here, is what the experiment aims for. The function of the image is exploratory because the image is central to the argument of the scientific paper wherein it is published: it then claims for authority[11]. In the second tier, reproducibility is unimportant and is indeed barely searched for, as it is often used only to confirm the presence or absence of an expected molecule, a confirmation technique[12]. The status of the image within the scientific argumentation is thus peripheral: it is only one step within a broader argument.

As a matter of fact, traceability is, in second tier electrophoresis, more important than actual replicability for editors of journals guidelines, according to Frow: "by providing details about how an image was acquired, transformed, and prepared for publication, the authors of the article acknowledge in writing their responsibility for its production" (Frow, 2014, p. 257). In a nutshell, trust in images is based on comparability in the first tier and traceability in the second.

[11] "Typically what we do is to put an image of a gel, carefully quantify it, but we have to repeat the experiments: it's an important parameter. It needs to be repeated 3-4 times, each time we will quantify it and the graph will show the mean value of the results with a standard deviation. This way you get the image that illustrates the quality of the results [...] and indicates under [the image] how many times the experiment has been repeated, and the results of the quantification. For example, in published papers, we can be asked to put the three [gel images] in annexes upon submission, to put all three or four raw gels to prove what we have done, that it's not invented." [Experienced professor in the academic sector]

[12] "If the goal is to analyze the protein, [...] we will choose techniques that make more sense rather than SDS-PAGE [for publishing]. […] It once was a technique that could give you a lot of information. But today, it's a simple technique that everybody uses, it's not revolutionary anymore." [Experienced researcher in the academic sector]

4. Conclusion: disciplining in retrospect

Through a diachronic narration of the evolution of the electrophoresis family of techniques (1980s-2010s), we have separated this family into two tiers. It is a story of a "digital turn" in the production of scientific images but, beyond a simplistic vision of the consequences of this turn, we highlight different epistemic statuses of the digitally produced images and consequently different meanings of reproducibility in these two tiers.

Within tier 1, digitization is linked to an aim for quantification and automation offered by gel-doc apparatuses, thus providing standardization. In turn, this standardization is underpinned by "collective disciplining", with an emphasis on comparability as a virtue for reproducibility. Within this framing, digitization then appears as "naturalized", and even compatible to an ideal of objectivity for practionners.

Within tier 2, digitization led to a change in (tacit) norms. Benchtop practices of image production in analog times that were once unnoticed or even invisibilized and yet collectively negotiated turned into problematic ones when they became, in digital times, desktop practices without any collective training or agreement. Analog practices that once were consensual and tacitly shared are then being replaced with digital practices that unsettle the ideal of "mechanical objectivity". In this regard, traceability, not comparability, is here the virtue required to achieve trustworthiness. Trust is gained all the more uneasily within tier 2 because its reproducibility virtue does not align with what Leonelli (2018) called an overarching "gold standard" vision of reproducibility, unlike within tier 1.

Returning to the Voinnet affair and similar scandals, we argue that these are idoneous to expose tensions regarding the epistemic role of images, tensions that are otherwise invisibilised. Unlike tier 1, no disciplining of digital practices is observed in tier 2, thus entailing a propension to distrust images in the community. Yet, Cambrosio and Keating (2000) aptly mention that collective disciplining also implies the definition and sanction of what is regarded as deviant practices within a scientific community. In that sense, affairs like Voinnet are a symptoma of a kind of collective disciplining *in retrospect*. This disciplining then sanctions practices that are thus regarded as deviant. Fabricated images at the heart of these affairs belong to a certain epoch and a certain epistemic context. What is striking in Voinnet and similar scandals is that gel electrophoresis images that are a decade old are today shamed online on a daily basis. This isn't

to say that some of these image processing practices are not indeed questionable or even sometimes outright cheating, but the community is experiencing a curious retrospective effect of disciplining of deviant practices.

This shaking of the community is amplified by a general feeling of distrust in times of reproducibility crisis. We argue that a general discourse on reproducibility as "one size fits all overarching gold standard" (in Leonelli's terms) does not take into account a diversity of epistemic practices: tier 1 gel electrophoresis is more fit to adhere to a so-called gold standard that insists on statistical relevance as an ideal of objectivity. Tier 2 electrophoresis images, on the other hand, have an epistemic status that is less fit to that "gold standard". It is probably not a coincidence that this retrospective effect concerns this particular field and this particular technique today. What has changed is the advent of post-publication peer-reviewing in platforms such as PubPeer[13]. Among a variety of scientific comments, some PubPeer posters focus on gel electrophoresis images of articles published long ago, with sometimes very little interest for the scientific argumentation within the targeted publication. Those typical second tier images are extracted from their publication context and are re-analyzed in the framework of picture forensics. Again, this is not to say that fraud does not exist or is not important.

A hypothesis to be explored in the future would be to look at whether exposing practices of published papers as ‘inappropriate’ on PubPeer – perceived as outright shaming by critics of the platform – could be seen as the application of a vision of overarching reproducibility to practices that do not fit well with this overarching vision. We focused in this paper on how scientific image production evolved from the vantage point of authors, but this opening could tell another side of the story, one of the changing readership of scientific images, the next step to better understand this crisis of trust.

[13] PubPeer is a grassroots web platform launched in 2012, whose purpose is to potentially open a discussion forum about any scientific article (Hocquet, 2020). PubPeer is a form of post-publication peer-review. It is radical in the sense that anyone can comment, and can even comment anonymously. The article review itself is thus becoming a live process. In times of crisis, it may even turn into a bursting of interactions. PubPeer is sometimes criticized among scientists for its anonymity and for its denunciatory atmosphere. In this regard, PubPeer functions like a social network. As Dubois & Guaspare (2019) point out, the tension lies in what constitutes a “peer”. Another criticism of PubPeer is the pervasive obsession of contributors about technical details (such as image editing) rather than the substance of the articles. What happens on PubPeer is also performative: contributors play the role of moral entrepreneurs by defining through commentary what is acceptable or deviant.

References

Amann, K. & Knorr Cetina, K. (1988). The fixation of (visual) evidence. *Human Studies*, 11(2), 133–169. https://doi.org/10.1007/BF00177302

Appel, R. D., Vargas, J. R., Palagi, P. M., Walther, D., & Hochstrasser, D. F. (1997). Melanie II - a third-generation software package for analysis of two-dimensional electrophoresis images: II. Algorithms. *Electrophoresis*, *18*(15), 2735–2748. https://doi.org/10.1002/elps.1150181507

Arentz, G., Weiland, F., Oehler, M. K., & Hoffmann, P. (2015). State of the art of 2D DIGE. *PROTEOMICS - Clinical Applications*, *9*(3–4), 277–288. https://doi.org/10.1002/prca.201400119

Beckett, P. (2012). The Basics of 2D DIGE. In R. Cramer & R. Westermeier (Eds.), *Difference Gel Electrophoresis (DIGE): Methods and Protocols* (pp. 9–19). Humana Press. https://doi.org/10.1007/978-1-61779-573-2_2

Cambrosio, A., & Keating, P. (2000). Of Lymphocytes and Pixels: The Techno-Visual Production of Cell Populations. *Studies in History and Philosophy of Science Part C: Studies in History and Philosophy of Biological and Biomedical Sciences*, *31*(2), 233–270. https://doi.org/10.1016/s1369-8486(99)00037-0

Chiang, H. H.-H. (2009). The Laboratory Technology of Discrete Molecular Separation: The Historical Development of Gel Electrophoresis and the Material Epistemology of Biomolecular Science, 1945–1970. *Journal of the History of Biology*, *42*(3), 495–527. https://doi.org/10.1007/s10739-008-9169-5

Dale, G., & Latner, A. L. (1969). Isoelectric focusing of serum proteins in acrylamide gels followed by electrophoresis. *Clinica Chimica Acta*, *24*(1), 61–68.

https://doi.org/10.1016/0009-8981(69)90141-7

Daston, L., & Galison, P. (2007). *Objectivity*. Zone Books.

De Chadarevian S., & Rheinberger H. J. (2009). Introduction. *Studies in History and Philosophy of Biological and Biomedical Sciences*, 40, 4–5.

Dowsey, A. W., Dunn, M. J., & Yang, G.-Z. (2003). The role of bioinformatics in two-dimensional gel electrophoresis. *PROTEOMICS*, *3*(8), 1567–1596. https://doi.org/10.1002/pmic.200300459

Dubois, M., & Guaspare, C. (2019). “Is someone out to get me?”: la biologie moléculaire à l’épreuve du Post-Publication Peer Review. *Zilsel*, 6(2), 164–192.

Dunoyer, P., Lecellier, C.-H., Parizotto, E. A., Himber, C., & Voinnet, O. (2004). RETRACTED: Probing the MicroRNA and Small Interfering RNA Pathways with Virus-Encoded Suppressors of RNA Silencing. *The Plant Cell*, *16*(5), 1235–1250. https://doi.org/10.1105/tpc.020719

ETH Zurich. (2015). Conducted properly – published incorrectly. Consulted online, 15 May 2022: https://ethz.ch/en/news-and-events/eth-news/news/2015/07/investigation-result-voinnet.html

Frow, E. K. (2012). Drawing a line: Setting guidelines for digital image processing in scientific journal articles. *Social Studies of Science*, *42*(3), 369–392. https://doi.org/10.1177/0306312712444303

Frow, E. K. (2014). In Images We Trust? Representation and Objectivity in the Digital Age. In *Representation in Scientific Practice Revisited*. The MIT Press. https://doi.org/10.7551/mitpress/9780262525381.003.0012

Gel slicing and dicing: A recipe for disaster. (2004). *Nature Cell Biology*, *6*(4), 275–275. https://doi.org/10.1038/ncb0404-275

Guasparre, C., & Didier, E. (2020). The Voinnet Affair: Testing the Norms of Scientific Image Management. In M. Biagioli & A. Lippman (Eds.), *Gaming the Metrics: Misconduct and Manipulation in Academic Research* (p. 157-167). The MIT Press. https://doi.org/10.7551/mitpress/11087.003.0016

Hocquet, A. .(2020) Open Science in Times of Coronavirus: Introducing the Concept of "Real-Time" Publication. *Substantia*, 4(1), Suppl. 1, 937. DOI: 10.13128/Substantia-937

Horgan, G., Creasey, A., & Fenton, B. (1992). Superimposing two-dimensional gels to study genetic variation in malaria parasites. *Electrophoresis*, *13*(1), 871–875. https://doi.org/10.1002/elps.11501301189

Kay, L. (1988). Laboratory Technology and Biological Knowledge: The Tiselius Electrophoresis Apparatus, 1930-1945. *History and Philosophy of the Life Sciences,* 10(1), 51-72.

Klose, J. (1975). Protein mapping by combined isoelectric focusing and electrophoresis of mouse tissues: A novel approach to testing for induced point mutations in mammals. *Humangenetik*, *26*(3), 231–243. https://doi.org/10.1007/BF00281458

Lemkin, P., Merril, C., Lipkin, L., Van Keuren, M., Oertel, W., Shapiro, B., Wade, M., Schultz, M., & Smith, E. (1979). Software aids for the analysis of 2D gel electrophoresis images. *Computers and Biomedical Research*, *12*(6), 517–544. https://doi.org/10.1016/0010-4809(79)90036-3

Leonelli, S. (2018). Rethinking Reproducibility as a Criterion for Research Quality. In *Including a Symposium on Mary Morgan: Curiosity, Imagination, and Surprise* (Vol. 36B, pp. 129–146). Emerald Publishing Limited. https://doi.org/10.1108/S0743-41542018000036B009

Lutin, W.A., Kyle, C.F. & Freeman, J.A. (1978). Quantitation of brain proteins by computer-analyzed two dimensional electrophoresis. In: Catsimpoolos, N. (Ed.), *Electrophoresis '78*. Elsevier, Amsterdam, 93-106.

Manovich, L. (2011). Inside Photoshop. *Computational Culture*, *1*. http://computationalculture.net/inside-photoshop/

Miura, K. (2001). Imaging and detection technologies for image analysis in electrophoresis. *Electrophoresis*, *22*(5), 801–813. https://doi.org/10.1002/1522-2683()22:5<801::AID-ELPS801>3.0.CO;2-X

Morange, M. (2020). *The Black Box of Biology. A History of the Molecular Revolution*. Harvard University Press.

O'Farrell, P. (1975). High resolution two-dimensional electrophoresis of proteins. *Journal of Biological Chemistry*, *250*(10), 4007–4021. https://doi.org/10.1016/S0021-9258(19)41496-8

Olson, A. D., & Miller, M. J. (1988). Elsie 4: Quantitative computer analysis of sets of two-dimensional gel electrophoretograms. *Analytical Biochemistry*, *169*(1), 49–70. https://doi.org/10.1016/0003-2697(88)90255-2

Piller, C. (2022). Blots on a field? *Science*, *377*(6604), 358–363. https://doi.org/10.1126/science.add9993

Porter, T. M. (1995). *Trust in Numbers: The Pursuit of Objectivity in Science and Public Life*. Princeton University Press. https://www.jstor.org/stable/j.ctt7sp8x

PubPeer. (2015). RETRACTED: Probing the MicroRNA and Small Interfering RNA Pathways with Virus-Encoded Suppressors of RNA Silencing. https://pubpeer.com/publications/15084715, accessed 05/22/2021.

Ramm, P. (1994). Advanced image analysis systems in cell, molecular and neurobiology

applications. *Journal of Neuroscience Methods*, *54*(2), 131–149. https://doi.org/10.1016/0165-0270(94)90188-0

Rasmussen, N. (1993). Facts, Artifacts, and Mesosomes: Practicing Epistemology with the Electron Microscope. Studies in History and Philosophy of Science, 24(2), 227-265.

Raymond, S., & Weintraub, L. (1959). Acrylamide gel as a supporting medium for zone electrophoresis. *Science*, *130*(3377), 711. https://doi.org/10.1126/science.130.3377.711

Reid, M. S., & Bieleski, R. L. (1968). A simple apparatus for vertical flat-sheet polyacrylamide gel electrophoresis. *Analytical Biochemistry*, *22*(3), 374-381. https://doi.org/10.1016/0003-2697(68)90278-9

Rheinberger, H. J. (2009). Recent science and its exploration: the case of molecular biology. *Studies in History and Philosophy of Biological and Biomedical Sciences*, 40, 6–12

Schlötterer, C. (2004). The evolution of molecular markers — just a matter of fashion?. *Nature Review Genetics*, *5*, 63–69. https://doi.org/10.1038/nrg1249

Shapin, S. & Schaffer, S. (1985). *Leviathan and the air-pump: Hobbes, Boyle, and the experimental life*. Princeton University Press.

Shapiro, A. L., Viñuela, A., & Maizel Jr., J. V. (1967). Molecular weight estimation of polypeptide chains by electrophoresis in SDS-polyacrylamide gels. *Biochemical and Biophysical Research Communications*, *28*(5), 815–820. https://doi.org/10.1016/0006-291X(67)90391-9

Smithies, O. (1955). Zone electrophoresis in starch gels: Group variations in the serum proteins of normal human adults. *Biochemical Journal*, *61*(4), 629–641. https://doi.org/10.1042/bj0610629

Ünlü, M., Morgan, M. E., & Minden, J. S. (1997). Difference gel electrophoresis. A single gel method for detecting changes in protein extracts. *Electrophoresis*, *18*(11), 2071–2077.

https://doi.org/10.1002/elps.1150181133