



## Ensuring Image Integrity When Reviewing Western Blots *Overcoming the Challenges Facing Editors and Publishers*

Western blots are frequently included in scientific papers to report results when analysing proteins in a sample. However, research shows that Western blot images are a common source of integrity issues, the publication of which can be harmful to journals. Here, Dr Dror Kolodkin Gal, founder of image checking tool, Proofig AI, explores the challenges editors and publishers face when reviewing Western blot images and suggests how to more effectively identify issues before publication.

Western blotting is commonly used in laboratories around the world to detect proteins and gauge their expression levels. A search for 'Western blot' on PubMed returns over 400,000 results, 20,000 of which are from 2022.<sup>1</sup> However, according to leading image data integrity analyst Jana Christopher, MA, the percentage of manuscripts flagged for image-related problems, including issues with Western blots, ranges from 20 per cent to 35 per cent.<sup>2</sup>

With potentially far-reaching consequences for any journals publishing such papers, and for the scientific literature as a whole, identifying and resolving these issues pre-publication is crucial.

### Risks to Publishers

The most serious consequence of publishing an article containing image integrity issues is retraction. Arguably, retractions have the biggest impact on the authors' careers and reputations, but the journal can also pay a price.

The process might begin with a reader privately writing to the author's institution to confess concerns about an article, or more publicly posting an anonymous comment on a platform such as Retraction Watch<sup>3</sup> or PubPeer.<sup>4</sup> The journal may then have to investigate, possibly in conjunction with other parties, such as the author's research institution, funding agencies or regulatory bodies. This process of investigating the results and the cause of the problem can take years.<sup>5</sup> Multiple retractions could also lead to journals being delisted from indexes<sup>6</sup> or closed.<sup>7</sup>

In addition, a retraction can damage the journal's reputation for publishing high-quality research, and reputational damage occurs during an investigation whether allegations turn out to be valid or not. This can lead to a loss of trust among readers, authors, and funding agencies. It can also make it more difficult for the journal to attract top-quality manuscripts in the future.

Retracting an article can also be expensive for the journal. It can involve the cost of notifying readers, authors, and funding agencies, as well as the cost of re-evaluating other articles by the same authors. In some cases, the journal can become

embroiled in legal battles or be forced to pay damages to the affected parties.

### Where Issues Arise

People reporting instances of image issues often assume that the only reason for altering an image like a Western blot is to falsify results or fraudulently increase chances of publication, but most issues are honest mistakes.

In my experience of working with researchers to check images in their manuscripts before submission, around one in four manuscripts includes at least one image integrity issue. Most of these issues are mistakes, like duplications, but even if a mistake is made innocently or because of disorganisation, it can still impact the conclusions of the research and be very problematic.

While rare, some alterations are a deliberate attempt by cheats to mislead the reader and increase the chances of publishing a manuscript. "Western blots are easy to manipulate in an unsophisticated way," said Chris Graf, Research Integrity Director at Springer Nature. "Researchers or paper mills might do that to beautify the data... or to fake it in the first place."<sup>8</sup>

### Volume of Submissions

The sheer number of submissions journals receive makes ensuring integrity and proactively identifying these issues a challenge. Many journals receive far more articles than they can publish. Science, for example, received over 10,000 submissions in 2022 and accepted just 6.1 per cent of them.<sup>9</sup>

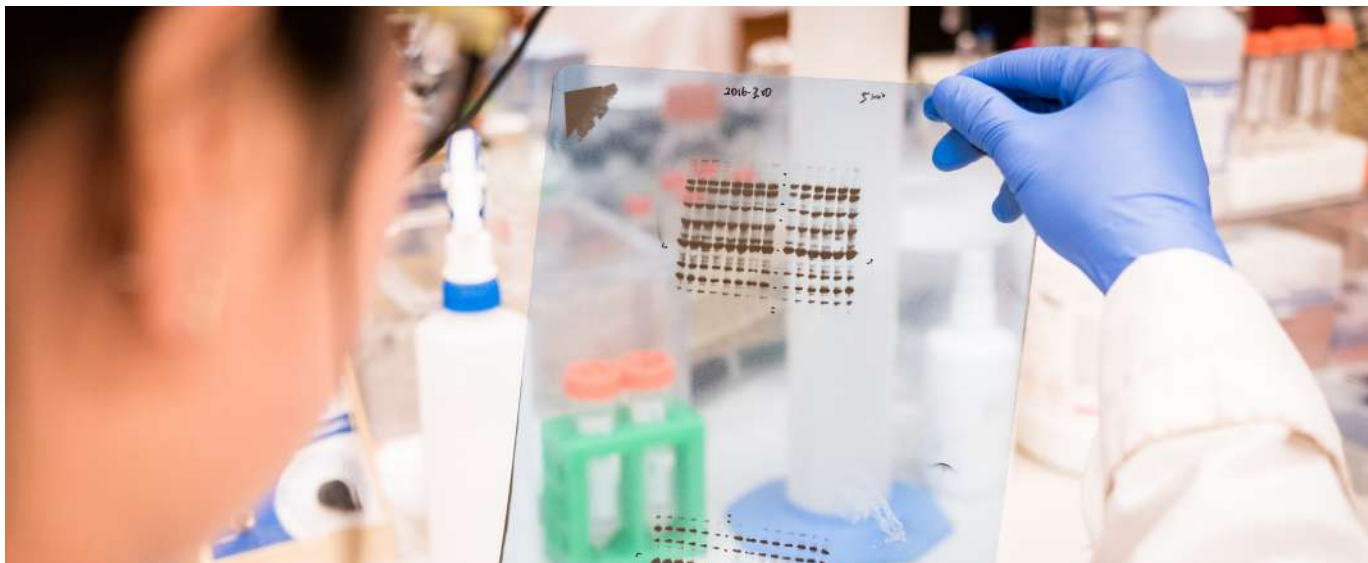
Say a manuscript contains images of 50 Western bands. Reviewing those 50 bands for simple duplications requires 2,500 separate side-by-side comparisons. Manuscripts often contain dozens of subimages, sometimes hundreds, so just reviewing the figures for duplication errors requires a substantial time investment, let alone reviewing the study's content or checking for less obvious image issues. Editors, reviewers and integrity teams therefore face a significant challenge in thoroughly examining this volume of work.

The challenge is compounded by the delicate patterns and subtle variations in band intensities, which often make Western blots look very similar to one another when reviewing by eye.

Proactive checks before publication offer significant benefits to journals and editors compared to reactive investigations. Streamlining checks prior to publication helps prevent problematic figures entering the literature, saves time and money and protects a journal's reputation, but doing this manually is often unsustainable.

### Automation

Using AI tools can significantly improve pre-publication image integrity checks by screening images and flagging any that require more detailed attention by a human reviewer.



Once images are uploaded to the platform, the tool scans them all, checking each first against itself and then all other images. AI tools can identify a range of issues, including duplication, rotation, flipping, cropping, splicing, and manipulations including deletion and insertion of bands. Any potential issues are then highlighted and flagged for the user, who can then investigate the tool's findings and make a final decision about whether or not an issue has occurred.

As well as reducing the time image checks take by selecting images most likely to contain issues, AI tools can also improve the investigation process. By using advanced filters to highlight anomalies that are invisible to the human eye, AI tools help catch errors that reviewers might have otherwise overlooked. This can enhance the overall accuracy of the image integrity assessment.

In a trial of image checking tool, Proofig AI, by the American Association for Cancer Research (AACR), tool-assisted review of manuscripts that had been provisionally accepted for publication identified more than twice the number of issues in just over half the time compared to manual review alone.<sup>10</sup> Such tools help editors and reviewers work more efficiently and allocate more time to other critical aspects of the peer-review process.

"There's the opportunity to use technology to interrogate [Western blot] images and to identify the overlapping regions within the image, the scrub marks that have been left by the use of an eraser tool in Photoshop, for example, and we're able to spot the fact an image has been manipulated," added Graf.<sup>6</sup>

These tools are not designed to replace human reviewers or to pass judgement. They offer time savings and efficiency improvements by highlighting potentially problematic images for editors to check in more depth.

While researchers are responsible for submitting work that conforms to the highest standards of scientific rigour, the probability of including problematic figures in journal publications is extremely high without prior examination using specialised software. Implementing AI tools for pre-publication

image analysis can assist journals in upholding their credibility, conserving resources and safeguarding the integrity of scientific literature.

## REFERENCES

1. <https://pubmed.ncbi.nlm.nih.gov/?term=western+blot>
2. UKRIO. Expert interview with Jana Christopher, MA, Image Data Integrity Analyst. [accessed January 21, 2024]. <https://ukrio.org/ukrio-resources/expert-interviews/jana-christopher-image-integrity-analyst/>
3. <https://retractionwatch.com/>
4. <https://pubpeer.com/>
5. <https://doi.org/10.1111/anae.14414>
6. <https://retractionwatch.com/2023/03/21/nearly-20-hindawi-journals-delisted-from-leading-index-amid-concerns-of-papermill-activity/>
7. <https://retractionwatch.com/2023/05/02/hindawi-shuttering-four-journals-overrun-by-paper-mills/>
8. <https://www.researchinformation.info/feature/how-do-we-improve-peer-review>
9. <https://www.science.org/content/page/journal-metrics>
10. <https://peerreviewcongress.org/abstract/use-of-an-artificial-intelligence-based-tool-for-detecting-image-duplication-prior-to-manuscript-acceptance/>



### Dr. Dror Kolodkin Gal

Dr. Dror Kolodkin-Gal, Ph.D. is a virologist and life sciences researcher that specialises in new *ex-vivo* explant models to help understand disease progression and treatments. During his research, he became familiar with the issues surrounding image duplication and image errors in scientific publications. Dror co-founded image check software provider Proofig to help colleagues avoid unnecessary reputational damage and the financial implications of an investigation into their careers, as well as academy institutions and publishing houses. Dror and his teams created Proofig's artificial intelligence algorithms to help detect image duplication and manipulations in scientific papers. The software checks papers prior to submission and publication, preventing unexpected rejections and helping to improve article quality and credibility.