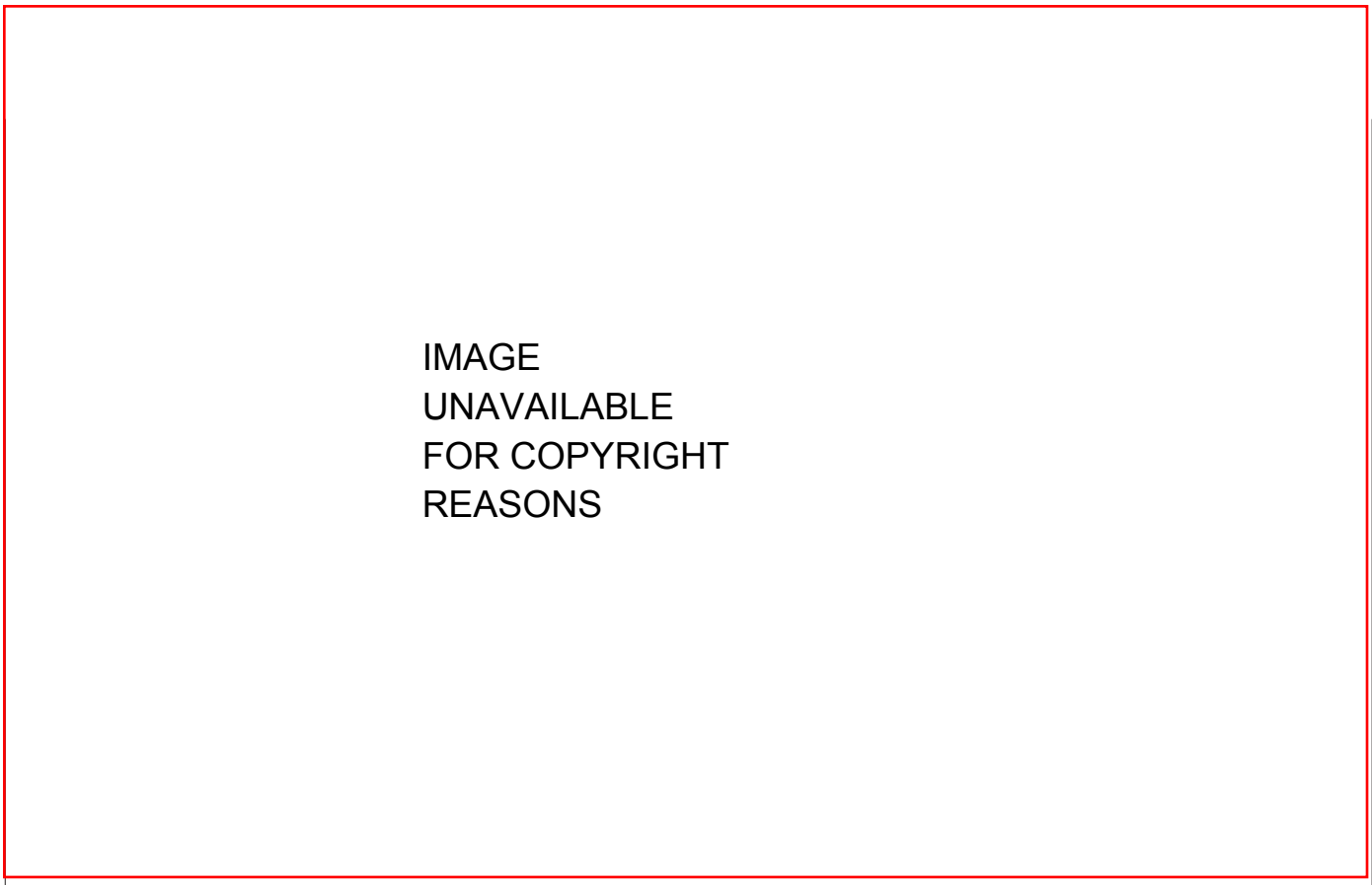

S. HUFFAKER/GETTY IMAGES

CSI: cell biology

Digital photography and image-manipulation software allow biologists to tweak their data as never before. But there's a fine line between acceptable enhancements and scientific misconduct. Helen Pearson investigates.

In a cramped office in midtown Manhattan, a forensics expert peers intently at a flickering computer screen. The shadowy image, hugely magnified, reveals a tell-tale dark smear. Something about it, she can tell, is just not right…

It could come straight from the screenplay of the latest hit TV crime show. But, in fact, such scenes are playing out regularly at the sedate headquarters of Rockefeller University Press — where the images under scrutiny are those of cells and gels in papers accepted for publication in *The Journal of Cell Biology*.

Mike Rossner, the journal's managing editor, introduced the forensics procedure in 2002 to patrol a growing practice among cell and molecular biologists: that of manipulating their scientific images. His specially trained editor hunts for tell-tale lines or smudges that might reveal where inappropriate modifications have been made — and when found, Rossner asks the authors for their original data.

In the vast majority of cases, the perpetrators aren't willfully misrepresenting their results — rather, they are unaware that their efforts to achieve the cleanest images for publication have crossed the line of acceptability. Such ignorance is widespread, and for Rossner it underlines why his journal now subjects all accepted papers to image forensics. "My goal is to catch problems before we publish," he says.

The availability of digital cameras and image-manipulation programs such as Photoshop has made it all too easy for researchers to enhance the DNA bands on a gel or brighten up the images of cells snapped on a slide. Most alterations are harmless: researchers legitimately crop a picture or enhance a faint, fluorescently tagged protein. But in some cases, innocent attempts to clarify an image can erase valuable data or raise suspicions of fabrication.

Following Rossner's lead, other journal editors now scrutinize images more carefully than they used to. And researchers, some burnt by questions about their own photos' integrity, are beginning to patrol the activities of their lab members. "We all underestimate the amount of skullduggery that goes on," says Joseph Gall, who studies the structure of cell nuclei at the Carnegie Institution in Baltimore, Maryland.

Picture perfect

Biologists have always gone to great lengths to create beautiful pictures that best illustrate their data. Geneticists carry out multiple exposures of radioactively labelled DNA fragments separated on gels in order to create a crisp image. Cell biologists once worked through rolls of film to grab the perfect shot from a microscope. But with Photoshop, a few clicks of the mouse can transform a featureless black microscope snap into a starry vista littered with labelled proteins. "You can make up almost any image you want nowadays," says Tom Misteli, a cell biologist at the National Cancer Institute in Bethesda, Maryland.

Some biologists seem to succumb to this temptation. In 1989–90, only 2.5% of allegations examined by the US Office of Research Integrity, which monitors misconduct in biomedical research, involved contested scientific images. By 2001, this figure had jumped to nearly 26% (ref. 1).

But such miscreants are in the minority. The more pressing problem, say experts, is that innocent efforts to smarten or prettify images end up with unintended consequences. At the very least, biologists risk erasing potentially valuable information, such as

D. BECKER/SPL

low levels of fluorescently labelled protein swilling around a cell's cytoplasm. At worst, such manipulations can lead researchers to the wrong scientific conclusions.

In some cases, researchers may not even realize that they are significantly altering an image, particularly when the changes are made at the time a picture is taken, by adjusting settings such as the exposure on microscopes or digital cameras. Many scientists are oblivious to the consequences of such actions, because they have only a rudimentary knowledge of the sophisticated equipment involved.

But it is the use of Photoshop or similar software to alter an original picture that scientists and editors say is most troublesome. With these programs, researchers can quickly carry out an array of modifications, from slicing off the messy edges of an image to using sophisticated algorithms to sharpen the edges of a blurred image on a microscope slide.

No one wants to ban image manipulation outright. In cell-biology experiments, for example, researchers often have to adjust the relative intensities of red, green and blue fluorescent markers in order to show all three in a single image. Even drastic changes are sometimes considered tolerable if scientists spell out exactly what they did.

> "The temptation to modify images comes from the fact that you have to sell a clear-cut story." — Tom Misteli

But it is tough to draw a precise line between acceptable and unacceptable image manipulation. Few journals have explicit policies, and of those that do, *The Journal of Cell Biology* has the most stringent guidelines[2]. These allow alterations that are applied equally across an entire image, such as changes to contrast or brightness. They also permit some other corrections, such as adjusting the brightness of pixels in a certain range of colours — but only if details of the adjustment are spelled out. Changes to selected parts of an image, such as brightening one cell in an entire field or scrubbing out an ugly blemish, are prohibited.

Blurred vision

Rossner estimates that roughly 20% of accepted manuscripts contain at least one figure that has to be remade because of inappropriate image manipulation. In the vast majority of cases, the authors have made the changes innocently, and can provide acceptable images once the problem has been explained. In rare cases where the journal suspects misconduct, the authors' institutions are informed.

For their part, scientists say that they feel under pressure to produce faultless images to present convincing experiments that reviewers and editors want to publish. "The temptation comes from the fact that you have to sell a clear-cut story," Misteli says. Tweaking images is also seductive in a way that adjusting statistics is not, because of the natural human desire to create an aesthetically pleasing picture.

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Caught on camera: image enhancements designed to clarify fluorescently labelled cell structures can also wipe out useful data.

Cell-signalling researcher Shigemi Matsuyama is one biologist who discovered the pitfalls of Photoshop when the images in a 2003 *Nature Cell Biology* paper[3] were called into question. His study showed how a protein called Bax, which is involved in triggering cell death, is controlled by a second protein called Ku70. At the time, Matsuyama was excited about the publication, which was his first after setting up his lab at the Blood Research Institute in Milwaukee, Wisconsin.

But his elation turned to despair when a colleague in the field contacted him and *Nature Cell Biology*'s editors a few months later to point out some troubling features in several images. The pictures were of western blots, a technique in which a labelled antibody is washed over several protein samples and bands on the blot to reveal which of the proteins bind to the antibody. Enlarging several of these images revealed straight lines on either side of some bands, suggesting that they had been pasted together on a computer. "I was stunned," Matsuyama says. "I couldn't eat for almost a week."

The first author of the paper, postdoctoral researcher Motoshi Sawada, had used Photoshop to put together some of the western blot images. In one example, he cut out a band from one position on the blot and pasted it into a second spot. In another, he reused bands from one blot in two separate figures. Sawada says that he felt under pressure to produce the figures quickly, and that one of the changes was a genuine mistake. He adds that he made the others so that the figures were clear and easy to understand. "I thought that it was acceptable," he says.

In the frame: Mike Rossner has championed the fight against unwarranted image manipulation.

In the event, Sawada and Matsuyama went back to the original data, and were able to produce corrected figures that confirmed their original scientific conclusions. These were published in a 2004 corrigendum[4].

Image problem

Sawada's case seems to be fairly typical. Scientists and journal editors say that most questionable image manipulation can be traced to inexperienced students or lab staff who are unclear about what is allowable. "It's junior people tidying up the image and not realizing that what they're doing is wrong," says Richard Sever, executive editor for the *Journal of Cell Science*, based in Cambridge, UK.

But whoever the perpetrators are, and whatever their motivation may be, there is so far little consensus about how to curtail them. One way, researchers say, would be to teach budding biologists about the ethics of image-making during postgraduate courses. Another is for lab heads to be more rigorous about policing inexperienced lab members. Matsuyama, for one, now demands that his students and postdocs show him the original image alongside the final form that they want to submit for publication.

Journal editors are also recognizing that they have a role to play. The *Journal of Cell Science* plans to draw up image manipulation guidelines for its authors within the next three months; *Nature Cell Biology* is encouraging researchers to submit original images of gels alongside the edited ones, for publication as supplementary information[5]. Another option would be for journals to demand that authors list the image adjustments they make in methods sections or figure legends.

Despite their heightened level of scrutiny, editors still fret that their forensic efforts will be outpaced by rapid advances in image-manipulation software, and researchers' skill at using it — the minority of biologists who are deliberately bending the rules are only likely to get better at covering their tracks. "We're just fortunate that most students and postdocs are not that good in Photoshop yet," Rossner says. ■

Helen Pearson reports for *news@nature.com* from New York.

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