

14-013099-CZ

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EXHIBIT B
to PubPeer's Motion to Quash

Affidavit of Dr. John W. Krueger

STATE OF MICHIGAN
IN WAYNE COUNTY CIRCUIT COURT

FAZLUL SARKAR,

Plaintiff,

Case No. 14-013099-CZ

vs.

Hon. Sheila Ann Gibson

JOHN and/or JANE DOE(S),

Defendant(s).

AFFIDAVIT OF DR. JOHN W. KRUEGER

I, John W. Krueger, being duly sworn, say as follows:

1. My name is John W. Krueger, Ph.D. For twenty years, from 1993 to 2013, I worked in the federal government as one of the original Investigator–Scientists in the Division of Research Investigations (which later became the Division of Investigative Oversight), within the Office of Research Integrity (ORI) of the Department of Health and Human Services.

2. As explained more fully below, while at ORI, I was responsible for the forensic evaluation of scientific images. While there, I developed the tools that ORI used—and still uses today—to forensically evaluate the authenticity of scientific images.¹

3. I have been retained by counsel for PubPeer, LLC to offer my professional opinion on a series of comments made on PubPeer’s site concerning images that appear in research papers co-authored by Dr. Fazlul Sarkar, the plaintiff in this lawsuit. This examination

¹ See Forensic Actions for Photoshop <<http://ori.hhs.gov/advanced-forensic-actions>> (link to download the “actions,” and to the explanatory “READ ME” files concerning how and why the Actions work and advice as to application and interpretation of the results).

draws no conclusion about the effect of any irregularity found in an image or images upon the integrity of the reported science, nor about who might be responsible.

Executive Summary

4. Counsel for PubPeer provided me comments made on PubPeer’s website that made two general types of observations: (1) that sets of images in papers co-authored by Dr. Sarkar looked “similar” or “identical” to each other (some invited comparison without stating an opinion one way or the other), and (2) that images in the papers displayed evidence of other irregularities (such as splicing of selected data). The exact comments provided to me, along with the titles of the related research papers, are listed further below in the Resources section.

5. At ORI, I would frequently receive similar comments about images used in scientific research papers. Typically, the comments would also claim that the similarities or other irregularities were evidence that the images at issue were not “authentic”—in other words, either that they did not in fact depict the results of *separate* experiments (but had been reused, whether intentionally or inadvertently), or that they had been manipulated in an inconsistent way (for example, when data appeared to have been selectively spliced into or out of the some but not all of the results in a consistent fashion). My job, then, was to analyze the images to determine whether there was sufficient evidence to conclude that the images were *not* authentic. If so, I would recommend to ORI that it send the results of my analysis to the host institution or university where the research had been conducted, and ask the institution to obtain and review the original data to learn if the latter supported the questioned image. If the institution concurred with my assessment regarding authenticity, it would conduct fact-finding and a formal review (under its own procedures) to determine whether the irregularities were due to research misconduct.

6. Counsel for PubPeer asked me to conduct a similar analysis here: to determine whether the images discussed by the PubPeer comments show evidence they are *not* authentic—again, whether they show evidence that they might not in fact depict the results of separate experiments, or were instead reused or modified in an inconsistent way that would affect the interpretation of the experiment. I performed that analysis and forensic comparison for a total of 28 separate issues that were identified in 18 observations from PubPeer. (That review included (by my count) approximately 44 images extracted from 25 full figures.)

7. As explained in greater depth below, I concluded as follows:

- a. My initial assessment of each image or images was based on visual observations of the source image(s), which I obtained afresh from each journal. In all 28 issues, there was sufficient visual support—based on morphology (shape), location, orientation and relative intensity (darkness) of the features in question in the images—to conclude that the images or their components were not authentic (did not depict *different* experiments as they purported to) or that they contained other irregularities (such as inconsistent splicing of data).
- b. The online source material was not of the best quality, and so I tested my initial visual observation using independent forensic methods that are more sensitive in detecting characteristic features in the kinds of images at issue. These methods specifically employed two tools, or “Forensic Actions” for Photoshop, that I had pioneered, and which are available from the ORI. In all 28 cases, my forensic evaluations yielded even more

definitive evidence that strongly supported the conclusion that the images I analyzed were not authentic or contained other irregularities.

- c. In 1 of the 28 questions I examined, the more sensitive forensics revealed new and more discrete evidence that was different from that originally posed by the PubPeer comments, but which nonetheless supported the questioning of the specific data in that case.

8. The scope of my review was limited to the figures cited in the PubPeer comments that I reviewed. When, during the course of examining the panel of its data at issue, new anomalies were identified elsewhere in the same figure, I documented those results as well. Although not presented in this affidavit, I identified other anomalies in six of the figures.

Professional Background

9. Attached to this affidavit as Exhibit A is a true and correct copy of my curriculum vitae. I briefly lay out below the relevant experience documented therein.

10. My direct expertise in forensic image analysis stems from 20 years of relevant federal work in my second career, starting as one of the original Investigator–Scientists in the Division of Research Investigations (or later the Division of Investigative Oversight), Office of Research Integrity (1993–2013). In this position, I was responsible for the initial assessment of allegations of data falsification and also for the oversight of investigations into allegations of falsification of research. Both tasks involved a heavy commitment to forensic assessment of the evidence, either for the allegations (sometimes made ‘anonymously,’ meaning that ORI had no way to determine the source the allegation) for referral to institutions, or in the evaluation of the resultant institutional findings. This was one of the more interesting ‘silent’ jobs in science, as it provided many new opportunities. At ORI, I:

- a. Pioneered and developed *de novo* the image processing methods for forensic examinations, including ORI's Forensic Tools, which are available on the ORI website (see links below). These tools have been provided and used world-wide.
- b. Developed the interpretations of the results, and advised and supported Journal editors in these matters;
- c. Trained numerous others in these methods (including my ORI colleagues and numerous institutional officials and faculty members) who were doing the investigations, as well as journal production editors doing image screening;
- d. Was heavily involved in education of the community about these new forensic methods and their interpretation. (See links to articles, material about ORI's forensic tools, and list of presentations, in my curriculum vitae; any item is available upon specific request);
- e. Established the Image Forensics Lectures for Institutional Officials at ORI's RIO BootCamp program (BootCamps I–VII);
- f. Established and successfully maintained the Macintosh Computer Forensics and software support in ORI. As part of this responsibility I also laid out the group Forensics lab ("Harvey's room" at ORI);
- g. My experience included working closely with lawyers defending ORI positions regarding appeals of specific PHS findings to the HHS Departmental Appeals Board.

11. Just as important as the ORI experiences working actual cases, I have developed an expertise in the judicious interpretation of the results of testing questioned images in science. This skill stems from my first career, which culminated in running my own laboratory as an independent, NIH-supported bench researcher and senior faculty member at the Albert Einstein College of Medicine (1975–1993). Prior to ORI I obtained a Ph.D. in Biomedical Engineering from Iowa State University ('71); I then trained at Imperial College, London ('72), was a *locum* lecturer at the Royal Free Hospital School of Medicine, and then a postdoctoral fellow at the center for Bioengineering University of Washington in Seattle ('72–'75). At AECOM, I:

- a. Was a peer reviewer for multiple papers in cardiac cell physiology, and served as an expert reviewer for NIH site visits for four program projects.
- b. Taught medical undergraduates, graduate students and postdoctoral fellows, and ten New York Academy of Science summer research interns (i.e., high-schoolers).
- c. My laboratory pioneered the laser diffraction methods for studying contraction in the subcellular level in heart muscle, and first reported the contraction of the isolated heart cell. (The latter methods became a common tool in the pharmaceutical industry.)
- d. With an MD–Ph.D student, now director of Cardiology at the University of Pittsburgh; the laboratory pioneered successful application of a new method to study excitation-contraction coupling in the single heart cell, that has formed the platform for more advanced techniques by others.

- e. Because of the above I was an Established Fellow of the New York Heart Association and the Wunsch Fellow in “Biophysical Engineering,” and I received specific invitations to international meetings.
- f. I also generated two patents on micromanipulators and hydraulic control (US Patent Office #4,946,329 and #5,165,297) that received commercial attention.

Background on Image Analysis

12. A scientific image is simply a picture purporting to show that a test was carried out and that the test produced a certain outcome. In other words, a scientific image reflects real data.

13. The value of a scientific image does not stem from the image itself or even necessarily from its quality, but from the results of the underlying test it purports to depict. One way of thinking about this is to consider two separate photographic prints taken of the same family at a Thanksgiving dinner. One might be grainy and the other crisp; or perhaps one was printed in color, the other in black and white. If the question is whether Uncle Joe was present for Thanksgiving dinner, and dancing later with Aunt Rita, however, *both* may be equally valuable in answering that question.

14. The primary question in evaluating a scientific image is whether it is an *authentic* representation of the data it purports to represent. This question often arises in the context of two images that purport to represent separate records of *different* experiments, but which contain similarities that suggest that the images in fact depict the *same* experiment, or the same observational record. To evaluate that possibility, the images would be reviewed to determine whether they were in fact derived from the same experiment. For example, returning to my imagined Thanksgiving event, does one of my two pictures actually show Uncle Joe dancing

with someone wearing Aunt Rita's unusual dress but instead sporting Aunt Nelda's face? If so, the proper conclusion would be that one or both of the images are not authentic.

15. It is critical to recognize that it is not necessary for two images to be pixel-for-pixel matches in order to conclude that they represent a record of the same experiment. This is so because two image files derived from the same source may “travel different routes” to their destination towards separate publication or use in reporting research. For example, they may have been subjected to different forms of digital compression—such as JPG compression—which would introduce differences. They may also have been modified in separate ways. One might have been lightened to make it easier to view, and another might have been resampled by being shrunk horizontally to fit on the page. Different changes can be made by different parties and also be introduced during printing at separate Journals. To return to our hypothetical Thanksgiving dinner, an analogy might be as follows: Uncle Joe sends a digital picture of the dinner to the entire family; Aunt Rita prints out a 4x6 color copy; Aunt Nelda prints out an 8x10 black-and-white copy; and Uncle Max crops out everyone from the photo except himself and then prints it out. All three siblings would have images depicting the same, or portions of the same, event or “experiment.” But the three images would look superficially distinct: one would be small and in color, another large and in black-and-white, and the final depicting only a single person rather than an entire family. Additionally, the various recording devices or printers they used may have introduced other differences, such as dots or lines that do not relate to the underlying observation.

16. These blemishes are generally referred to as “artifacts” in the context of scientific images. They are especially significant in image analysis because they generally ought to be randomly distributed from image to image or, at least, randomly positioned with respect to the

data from independent experiments or events in time. (Do the two pictures of Uncle Joe show that both “Aunt Rita” and “Aunt Nelda” share the same context, i.e., are other couples dancing elsewhere in the same position in each picture?) When they are not randomly distributed or randomly positioned, especially with respect to the data, artifacts produce unquestioned support for concluding that two images with conflicting content actually depict the same experiment.

17. Again, pixel-for-pixel perfection is not necessary in order to conclude that two images depict the same experiment. Instead, the question is whether there are characteristic features unexpectedly in common between the images that indicate that they are “too similar to be different.”

18. Relatedly, it is important to understand that affirmative similarities between images are more determinative than differences. In other words, the similarities between features in two images may lead to the conclusion that they derived from the experiment, even if there are differences between the images. The chief uncertainty arises from a false negative (i.e., wrongly missing the similarity between two images) due to poor image quality, rather than false positives (incorrectly concluding that two unrelated images are the same data).

19. Below, I explain how scientists forensically evaluate images, including how they examine whether two images purporting to represent different experiments in fact represent the same experiment. Then, I explain how I applied that methodology to the various images in the papers co-authored by Dr. Sarkar commented upon by PubPeer’s users.

General principles of forensic image analysis.

20. In assessing whether a scientific image or its components are authentic or, instead, depict conflicting results of the same experimental observation, the question to be answered is

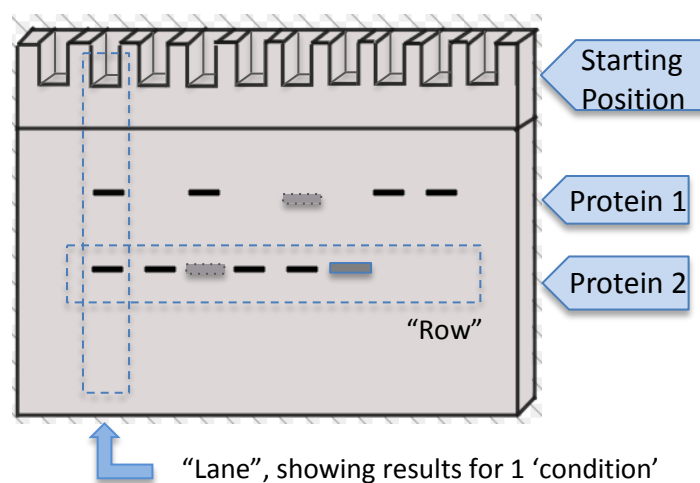
whether the content of each is “too similar to be different”—too similar, in other words, to have been derived from different experiments.²

21. The mode of analysis of the image will depend on the nature of the experiment being documented. In general, however, the images are compared to determine whether their contents share features that are unexpected to be common to each. This analysis includes any features in the images, their shapes, their position, the background noise, any artifacts, and, importantly, the relationship between two or more of the features. In comparing images, it is often very revealing to look at features that would otherwise not be noteworthy (nor of particular interest to the scientist who produced them), such as fine details hidden in the light background, or specific features buried in the dark bands. Artifacts can also be very revealing. An artifact in a scientific image is simply a feature of the image that results from the procedures being used rather than from the specimen under study. In common parlance, they could be thought of as blemishes or noise. What is important to science is the signal; what is important to forensics is the noise.

22. As explained above, two images need not be pixel-for-pixel matches in order to conclude that they depict the same experiment. This is because the test is not whether the *images* are the same object, but whether the images depict the same underlying *experimental observation*.

² An “image” in science is (1) a graphical representation of data (‘raw’ or ‘primary’ data); (2) that are the results of a unique experimental determination, reproducibly recorded by a device; and (3) that has intrinsic features that can reveal its lack of authenticity. Importantly, an “image” in science contains all the information needed to assess its inauthenticity because it is data that purports to be the product of a unique experimental determination. Thus, any question about a scientific image can be assessed, irrespective of whether the questioner is known or not. The image alone provides sufficient witness for its own worth.

23. Most of the images that I reviewed from the papers co-authored by Dr. Sarkar are images of so-called “Western blots.” A Western blot is an experiment that is widely used to study proteins because it allows researchers to detect specific proteins in the sample being studied. Very generally speaking, Western blots work by forcing the proteins from different samples through a “gel” (literally, a jelly-like substance sandwiched between two glass plates) using an electrical current so that the proteins separate, usually by their three-dimensional structure (larger proteins move more slowly through the gel) or by their polypeptide length (longer proteins move more slowly through the gel). Once the proteins are separated, they are then typically transferred from the gel to a membrane, where they are “stained” to allow them to be photographed. The picture below is a very simplified representation of a Western blot. The protein samples are loaded into the “wells” at the top of the gel, and the proteins then migrate down the gel in their respective “lanes” upon application of an electrical current. The end result (once transferred to a membrane, stained by using probes that make selected proteins visible, and photographed or otherwise recorded by an imaging device) is a unique pattern of “bands” showing how far each protein of interest migrated down the gel.



24. The individual lanes (10 shown here) permit testing the effect of a combination of different conditions upon *the amount* the protein of interest, as shown by the relative size and darkness (density) of its band. Typically, an image of a row of proteins of interest is selected for reporting the results. When the result of the same test is compared to its effect upon another protein, a new row will be selected. Obviously, the same lanes must be shown in both rows to interpret any differences.

25. Sometimes the effect of a test on multiple proteins is examined, but not all of the results prove to be needed. In this case the image of the rows can be cut and spliced together to rearrange their layout for a logical order of presentation. When this is done, all rows must include the same tests (say, those in lanes 1-4, and 6-9), and the splices must appear at the same position in all rows. Splice lines that differ from row to row can ‘de-authenticate’ a blot, because then the conditions for the respective tests can’t match.

26. When analyzing Western blots for authenticity, the same principles outlined above apply. The analysis looks to the features in the Western blot (the main features are typically the bands), their shapes, their position, their particular size/intensity (related to how much is protein is present), the background noise, any artifacts, and the relationship between the features. Artifacts in Western blots can take the form of distortions of the lanes, unusual features of the bands, faint boundaries of the blot, standards (or marker proteins for measurement), and even rulers placed on the blot for photography, etc. Enhancement may reveal faint characteristic features that were “hidden” in the lighter background around the bands, or even the inner details of single bands and their margins.

27. Some forms of artifacts might re-occur, such as those introduced by faulty equipment (for a Western blot, it might a faulty film dryer or the edge of a blot on an

autoradiographic film). The key question in cases of “replicating” artifacts is whether a fixed relationship to other features should exist? Thus, the key feature that makes an artifact determinative of inauthenticity is not its expected irreproducibility, but the fact that it should not be reproduced in the same relationship to independent features of the blots in two separate experiments.

Tools of forensic image analysis.

28. The first step in any image analysis typically involves visual observation of the image to determine whether there is cause for further examination. Visual observation relies on the human eye to detect the sorts of similarities discussed above that may be indicative that the images are not authentic, i.e., that they derive from the same experiment. An irregularity may not be initially perceived because it gets “lost in the crowd,” but after it is discovered it is often visually quite clear.

29. The second step involves forensic analysis of the images to determine whether the initial cause for concern is supported, or whether there might be additional evidence that can be detected. There are many tools to conduct such forensic analysis. The two discussed below are the ones that I used in analyzing the images from the papers co-authored by Dr. Sarkar and are ones that I pioneered in my time at ORI. They are freely available online and have been the primary tools that ORI uses in investigating claims that images are not authentic. They are useful because they provide a more sensitive way to visualize characteristic features in images for comparison. For Western blots, they allow a more sophisticated comparison of individual bands, artifacts, and background. They simply define the evidence in concrete terms so that the questions can be resolved.

30. There are invariably features in images that are hidden from human perception. The first tool, called “Advanced Gradient Map-Adjustment Layers,” promotes detection and

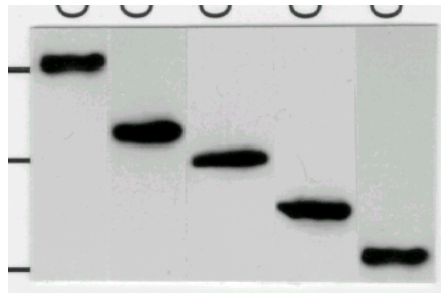
awareness of those features. This tool increases the contrast in, and then applies a false-colorization to, the image to help visualize the subtle features so that they can be compared. Its usefulness derives from the fact that human visual perception is limited. The eye, which responds by detecting contrast, can distinguish only about 50 shades of gray or fewer, but it can detect 100 shades of color. However, unlike the eye, a computer's ability to distinguish shades is not dependent on contrast in the image; it can selectively amplify very slight differences in shade. In addition, the small differences in shade that remain can be made further visible by converting them to different colors. In Western blots, enhancement of the small differences in shades (especially at the margins of features) can expose minute structural details in the morphology (shape) of bands, which otherwise would look smooth and featureless.³

³ This forensic tool works by (1) remapping the relation between the input to the output intensities, so as to extend the areas of high contrast, and by (2) false-colorizing the grey scale image (see "READ ME" files here: <http://ori.hhs.gov/advanced-forensic-actions>). Together, both effects promote detection of similarities by overcoming the physiological limitation of human vision to detect small differences in grey-scale images.

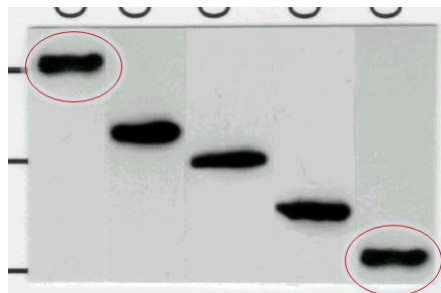
The false-color enhancement does not introduce new content to the image; rather it simply presents the same content in a different form, albeit at first appearing "strange." The latter action (false-colorizing) promotes perception of any features that are visualized by breaking down psychological factors leading to "confirmational bias."

In practice the rate of false positives is very low (so far, in my personal experience, it has not yet occurred). The approach is accepted by the scientific community and used by journals for pre-publication image screening; the method is available online and it can be easily explained without mathematics; when used with the adjustment layers in Adobe Photoshop, the results can be shared and precisely replicated and examined retrospectively without destruction of the tested image. They are available at <http://ori.hhs.gov/forensic-tool> and <http://ori.hhs.gov/advanced-forensic-actions>.

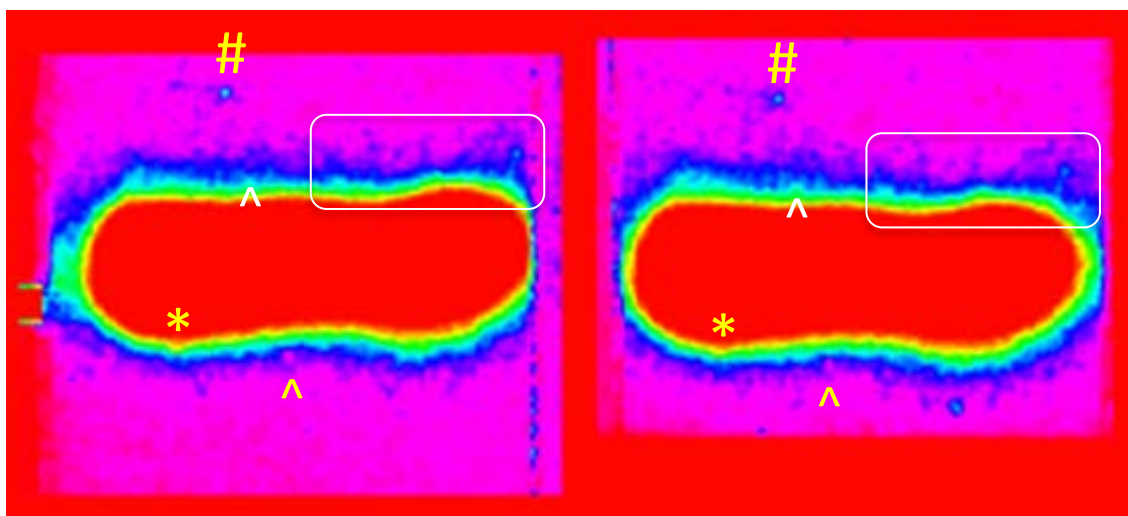
31. Here is an example from my time at ORI of the use of this first tool. This first image shows an image of a Western blot from a closed ORI case.



32. Concerns had been raised about the authenticity of the bands in the first and fifth lanes (the bands on the far left and the far right):



33. The two bands were subjected to the Advanced Gradient Map-Adjustment Layers tool, yielding these two images, shown side-by-side for comparison (with my annotations):



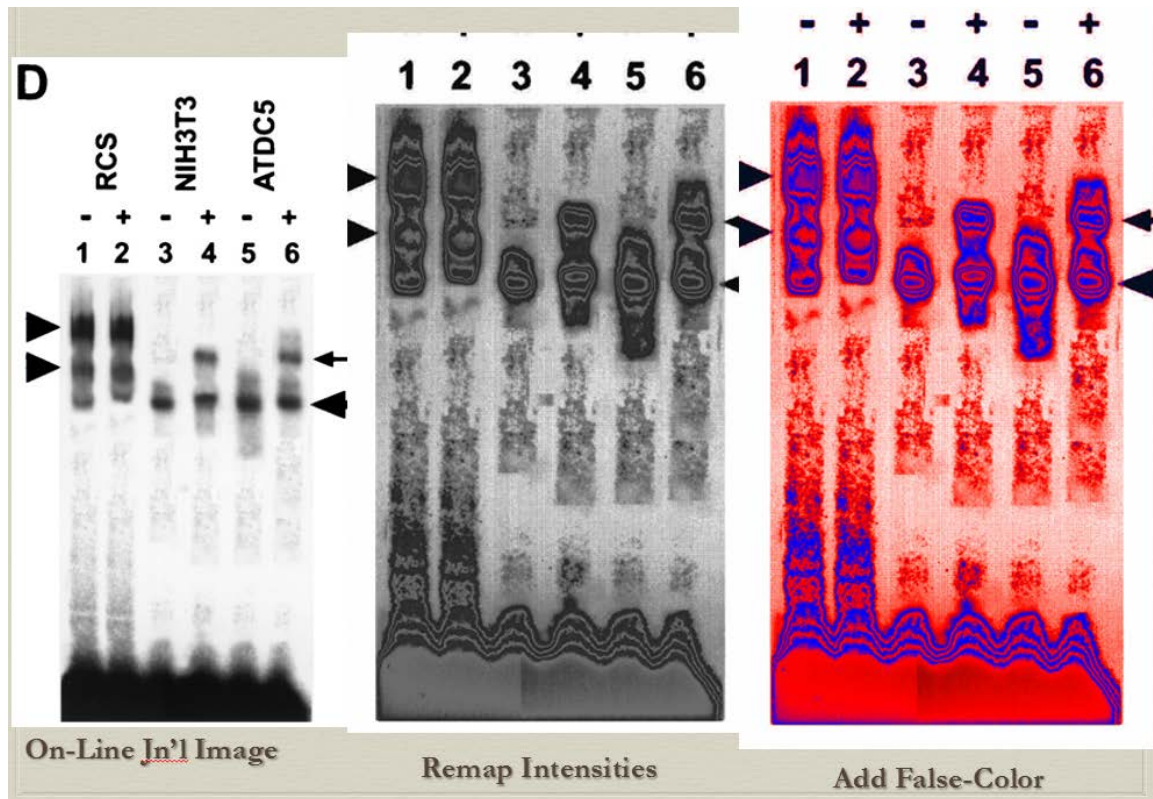
34. Contrast enhancement and false-coloring of the image demonstrated that the respective bands share similar miniature features, features which ought otherwise be random and unique to each band. For example, note the similarity in the morphology of each band, as shown by the inner margin of its red interior. Note, too, the similar artifacts above and on the right side of each band, and the blue spot denoted at #. And note other similarities along the margin of the band. The demonstration of similarity is made more compelling because separate artifacts that exist are present in similar relationship—both to each other and to the band itself.

35. Close inspection can also identify some examples where the fine detail differs between the two images, but those differences could have been introduced by the copying of the data. (This example represented the pasting of a separate photographic print over the blot.) More important, any of the small features that are dissimilar here do not account for the fact that all features that are similar have the same spatial relation—both to each other and to the band. This illustrates also why the existence of affirmative similarities are always more significant than pixel-to-pixel differences.

36. This image analysis showed that the first and the last lanes, purported to be different in origin, actually were from the same experiment. As should be obvious, the question is solely whether the images are too similar to show the results of different experiments. The differences may have arisen from different handling of the bands or the image compression applied to them, while the similarities and their position would not have done so.

37. Here is another example of the application of the first tool to show forensic detail in the background, and within the band itself. (Image from a closed ORI Case) Note, in particular, the similarities between the backgrounds (very easily visible with the false color) of each of the lanes. Those backgrounds ought to be random and relatively featureless, and yet clear

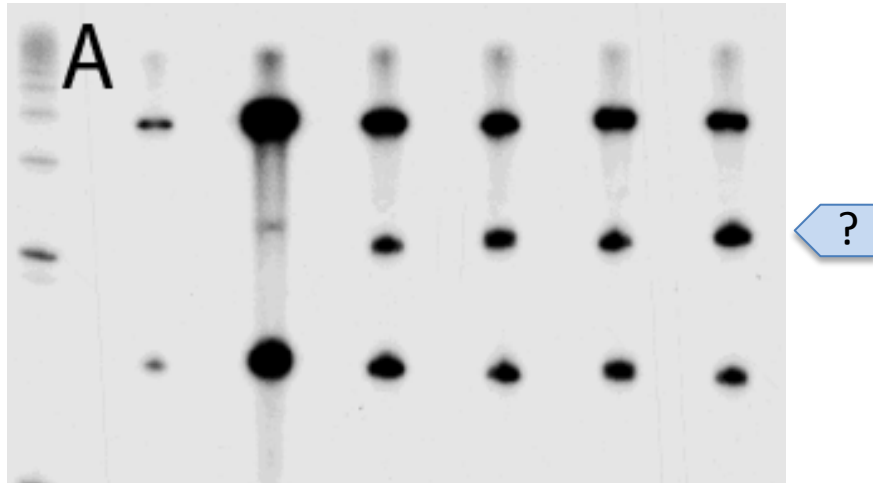
evidence exists of reuse and sharp boundaries where none should exist. Note, too, the similar morphology and internal structure of each of various pairs of the bands. For example, the uppermost bands in lanes 1 and 2 look unexpectedly similar in the false-color image, as do the components of bands that are side-by-side in lanes 5 and 6 (the band in lane 5 appears vertically stretched as compared to the band in lane 6).



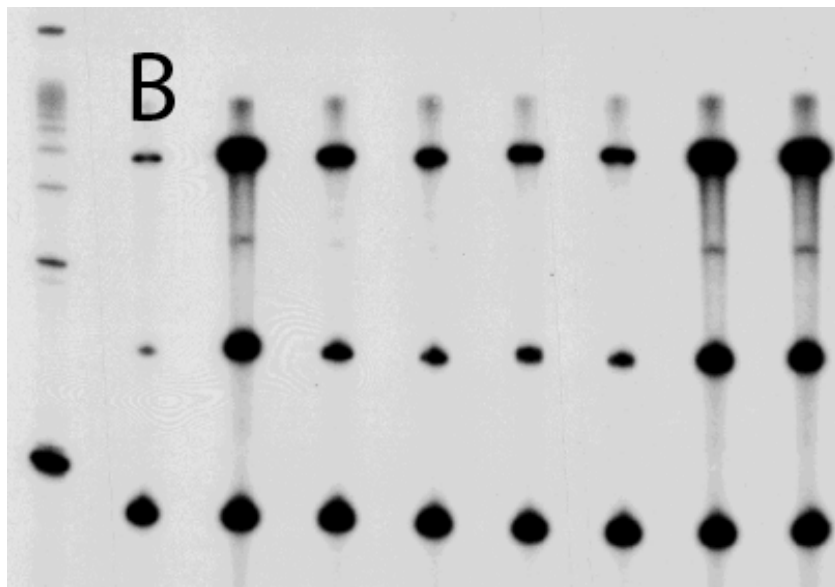
38. The second tool that I used in analyzing the images from Dr. Sarkar's papers is called "Overlay Features in One of Two Images." It works by overlaying two images in a way that allows both the visualization and the interpretation of their differences. The images are color-coded to identify from which image a disparate feature arose.⁴

⁴ The basis of the color-coded image overlay method to compare the shapes and features in two images is well accepted in science, being fully analogous to the approach widely used for the co-localization of proteins in cell biology. All forensic tools are available, along with "READ ME" advisory files, at <http://ori.hhs.gov/advanced-forensic-actions>.

39. Here is a final example, again from my time at ORI, of the use of this tool, an illustration that was developed for teaching Institutional Officials. This first image “A” is of a Northern blot (similar, for our purposes, to a Western blot) from one paper:

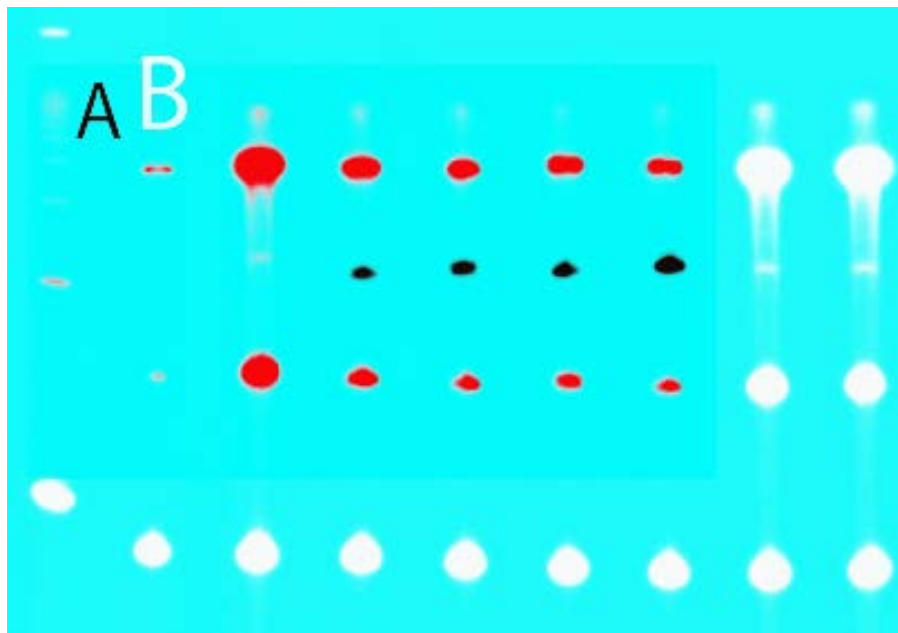


40. And here is a separate image “B” of a Northern blot from another paper:



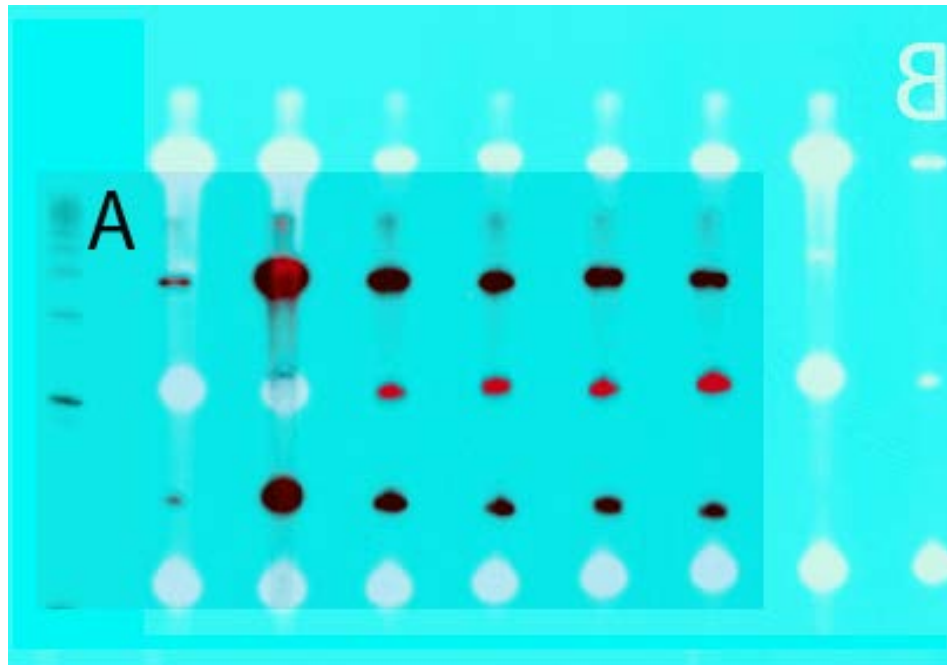
41. These two blots were designed to raise concern, because the two show evidence of similar origin: Specifically, the first two rows of bands in the second blot look suspiciously like the 1st and 3rd row of bands in the first blot, yet the first blot (Image “A”) has another row of bands between the rows that is not present in the second blot (Image “B”).

42. Whether the two conflicting images are from the same source can be tested using the overlay tool. Here is the result of such an overlay comparison to test the possible differences between the two images and to identify from which image they arose. In this overlay, differences from features that are derived only from the first image “A” appear black, differences from features that are derived only from “B” are white, and features from both images that overlap precisely appear red with uniform edges. (You can see this, starting with the color of the labels “A” and “B” in the original images.)



43. The overlay shows that 1st and the 3rd rows of bands in Image “A” are the same as the 1st and the 2nd rows in Image “B,” yet the other lanes are different. One of the images is not authentic data.

44. Here is a result of a second overlay of the same two images, but this time, the second image “B” has been flipped (*reversed*) horizontally (i.e., across its vertical axis) and repositioned to overlie its second row upon the second row of questioned data in the first image “A.” The color-coding for the similarity and the differences in the overlay is the same as before. The overlay comparison shows the questioned second row of four bands in “A” was fabricated by copying and reversing, and splicing into “A” the second row of data from “B.”



45. As before, red features *with uniform boundaries* denote overlap of the same bands (where the margins are not the same, they are different features (as seen in the second lane)).

46. These examples demonstrate 1) how image enhancements may extract more information from the content of an image than would be visibly apparent in a questioned image (i.e., points 28-37), and 2) how a comparison by direct overlay to reveal differences can be used to test the origins of bands in a questioned image (i.e., points 38-45).

Analysis of Images in PubPeer's Case

47. Counsel for PubPeer retained me to evaluate six sets of questions arising from eight papers co-authored by Dr. Sarkar. PubPeer's counsel provided me the text of the PubPeer comments relating to those six sets of questions, and I independently evaluated the images focused on by those comments to determine whether the evidence shows those images are not authentic. Collectively, those questions involved the examination of 28 separate issues, identified in 25 separate Figures of data in those eight papers.

Methodology.

48. My preliminary assessment was based on a visual inspection of the questioned images, provided either as PDF figures from the publication, and/or images obtained via PowerPoint slides of the relevant figure as downloaded from the journal.

49. Where possible, I conducted a more definitive examination using better-quality images that I was able to obtain from the journal's online image browser, using the "html" version of the paper. When possible, the images were expanded at the source using the journal's online image browser.

50. When deemed useful, I also tested each set of images using one of the independent forensic tools described above.

51. The primary issue I examined was whether individualized features in the separate images, the distinctive appearance of individual bands, and/or the related background, collectively were too similar to be the results of different experimental observations. In several images, I instead examined whether there was evidence of selective splicing or other irregularities demonstrating tampering with the image contents.

52. I concluded that there was sufficient reason to question the authenticity of the images I examined if any relevant similarities in the images could not otherwise be ruled out as being due to other factors.

Results.

53. As stated above, I concluded that, for each of the 28 image-issues that I evaluated, strong evidence supports the conclusion that the images are not authentic or contain other irregularities symptomatic of tampering. As also stated above, in one of the sets I examined, the more sensitive forensics revealed new and more discrete evidence that was different from that originally posed by the PubPeer comments, but which nonetheless supported the questioning of the specific data in that case.

54. I first based my opinion on my visual observations of material that I obtained directly from the journals. I concluded that sufficient reason existed to question the authenticity of the images.

55. Additionally I used a fully independent means of comparing the questioned images, one that visualized specific features in the morphological details of the bands and in amorphous features of the associated background. This approach provided a more sensitive means of evaluating the content “hidden” in same image(s), and it utilized the same sources that were available to the PubPeer commenters. That more sensitive approach fully supported my initial conclusion that the questioned images were not authentic, either because they were too similar to be different or because they showed evidence of inconsistent modification (e.g., splicing for one band that did not correlate with other bands in the same lane).

56. In one exception, however, the more sensitive examination found direct evidence for displacement of the questioned band from elsewhere in the image of the results (as opposed to its being copied and reused, a practice for which evidence was found in multiple other

images). Thus, even here, the question as to the authenticity of the band is fully sustained, but it is based on a different reason than that originally proffered.

57. Finally, the more sensitive methods that I applied detected *other* anomalies in the images occurring elsewhere in the same figures at issue. Collateral observations were associated with six figures.

58. Below, I explain my analysis in the context of a few examples from the 28 analyses that I conducted. These few examples are representative of my analysis and of my conclusions.

Examples of analysis of images from papers co-authored by Dr. Sarkar.

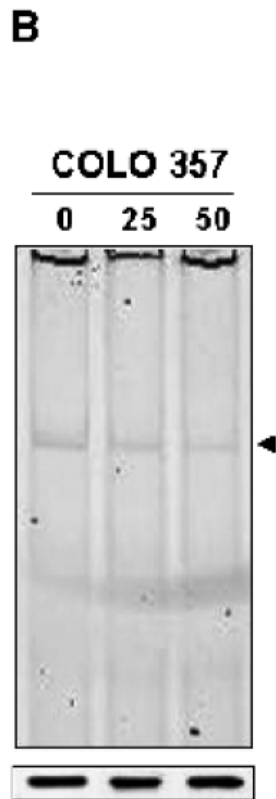
59. **First example.** The first example concerns images in the following paper published in 2005: *Molecular Evidence for Increased Antitumor Activity of Gemcitabine by Genistein In vitro and In vivo Using an Orthotopic Model of Pancreatic Cancer*, Sanjeev Banerjee,¹ Yuxiang Zhang,¹ Shadan Ali,¹ Mohammad Bhuiyan,¹ Zhiwei Wang, Paul J. Chiao, Philip A. Philip, James Abbruzzese, and Fazlul H. Sarkar.

60. The comment that PubPeer commenters made on the article, as provided to me by PubPeer's counsel, was as follows:

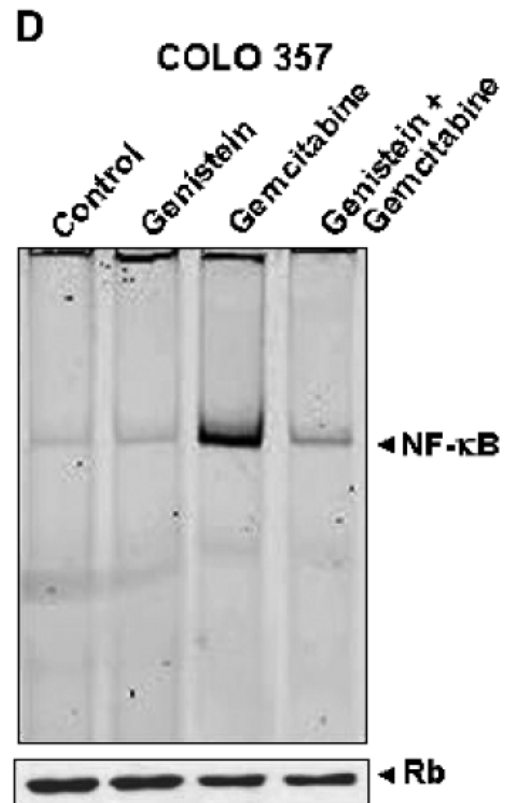
Compare Fig. 3B and Fig. 3D

When Colo357 lane for 0 and 25 in 3B is flipped it looks similar to the control and genistein in Fig. 3D for Colo357.

61. I examined Figures 3B and 3D to determine whether they show evidence of inauthenticity. Here are the Colo357 portions of each of the two figures as they appear in the journal article:



From Figure 3B



From Figure 3D

62. The comment calls for a comparison between the first two lanes of each portion of the figure, with the lanes from Figure 3B being flipped. I performed that flip, which resulted in the following comparison:

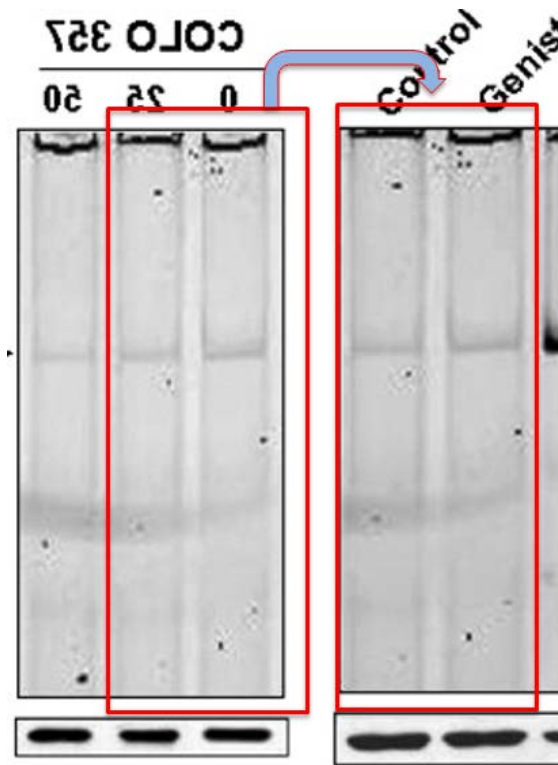
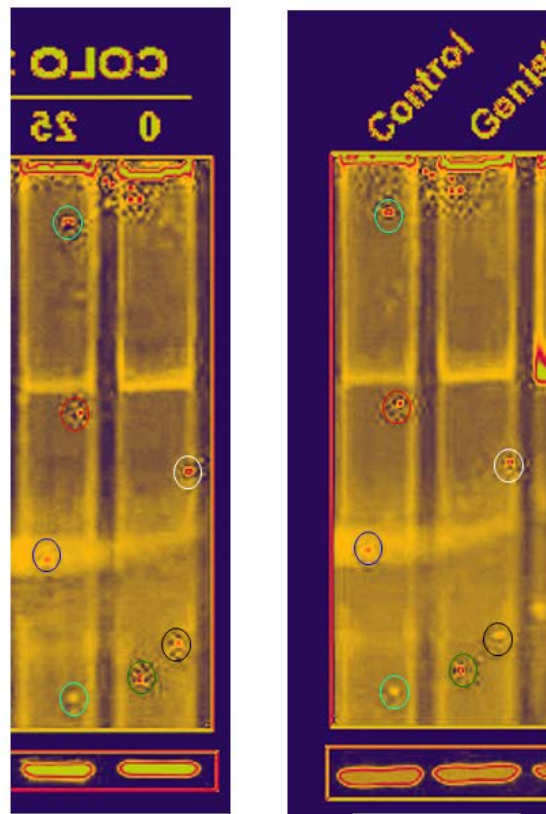


Figure 3B (flipped) vs. Figure 3D

63. Based on a visual inspection alone, there are multiple examples of artifact that are common both in appearance and in position, to both images. A visual inspection of the images is sufficient to conclude that there is strong evidence to believe that these images are not authentic.⁵

⁵ Note that the lanes in each figure appear to be of different widths. As I explained before, differences are less revealing than similarities, and the different widths do not alter my conclusion. It is common for researchers to shrink or expand their results to fit the layout of a new figure, or to allow easier comparison across experiments. Journal art editors also do this during printing.

64. I repeated the comparison using images directly obtained from the journal. The dynamic range of the features visualized was extended through false-color enhancement. As explained above, such enhancement visualizes features in both the background and in the random noise that occurs in common between the two panels. The enhancement further confirmed that the respective features in each image are all in the same position relative to each other. This strongly confirmed the visual inspection.



65. Note that the small circles in the false-color image above were added on top of one of the images, grouped together, copied, and then overlain on the second image. They show that the relative position of the artifacts, both with respect to each other and with respect to the experimental results, are the same.

66. One might ask whether the possibility exists that the multiple artifacts are in the same position because they were present on a device used to record different sets of data? What

establishes the significance of the artifact, however, is not its presence, but the similarity of its relationship with experimental results, a relationship that should vary in the repositioning of new results when making an independent record of their observation.

67. Thus multiple artifacts that ought to be randomly located occur in the same relative position in two images. Despite this expectation, the artifacts are also in the same relation to the layout of the blot's lanes, and to its band position. The latter agreement proves that the two images cannot be separate results from independent experimental determinations.

68. **Second example.** The second example concerns images in the following paper published in 2006: *Down-regulation of Notch-1 contributes to cell growth inhibition and apoptosis in pancreatic cancer cells*, Zhiwei Wang, Yuxiang Zhang, Yiwei Li, Sanjeev Banerjee, Joshua Liao, and Fazlul H. Sarkar.

69. The comment that PubPeer commenters made on the article, as provided to me by PubPeer's counsel, was as follows:

Figure 1D

UPPER Notch-1 panel: please compare NS of BxPC3 (lane 2 from left) with NS of HPAC (lane 4 from left) and CS of PANC-1 (lane 5 from left). Note also the vertical line and darker background on the left side of the CS band of PANC-1.

LOWER Notch-1 panel: please compare CP of HPAC (lane 3 from left) with CP of PANC-1 (lane 5 from left). Also compare the CP band of BxPC3 (lane 1 from left) with the NP band of PANC-1 (lane 6 from left).

Now, please FLIP HORIZONTALLY the entire LOWER Notch-1 band. Now compare the NP band of BxPC3 in the lower Notch1 panel (lane 2 from left in the original) with the CS of BxPC3 in the upper Notch-1 panel (first lane from left). Also compare the CP bands of HPAC and PANC-1 in the lower Notch-1 panel with the NS bands of BxPC3 and HPAC in the upper Notch-1 panel.

Figure 5

Cyclin D1 Panel: please compare the shape and position of the CS band of HPAC with the CS band of PANC-1 in the Cyclin D1 panel (upper).

CDK2 Panel: please note the vertical line between the NS band of HPAC and CS band of PANC-1. Please note the box around the NS band of BxPC3 (magnify).

Figure 6A, B and C

Please compare the Rb bands in the three panels (A, B, and C). Compare the BxPC3 and HPAC bands in 6A and 6B, magnify and see the shapes and background, especially the small specks in the upper right corner of the second band (from left). Now, please FLIP HORIZONTALLY the RB bands in PANC-1 (panel C) and compare with the two other bands (BxPC3 and HPAC in panes A and B). Then, note the small specks in the upper right corner of the second band (from left).

Figure 7E and Figure 8D

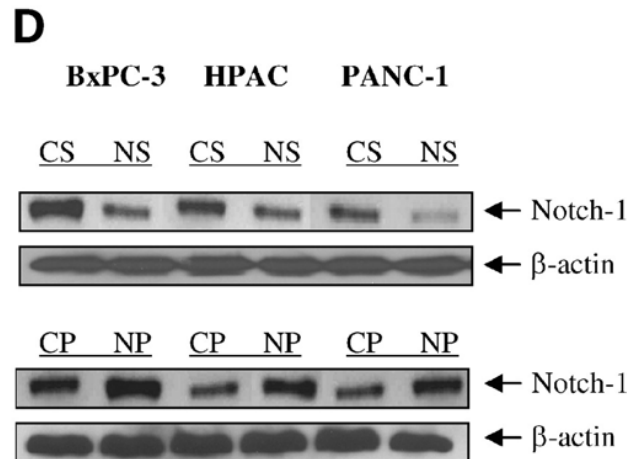
Please compare the two Rb bands. But please increase the width of the Rb bands in Figure 8 and compare. Better seen in PowerPoint, magnify.

70. A comment related to the same paper, comparing a figure from it to a figure from another paper, was as follows:

Fig. 8A in this paper is identical to Fig. 5A in Cancer, 2006 Jun 1;106(11):2503-13; (<https://pubpeer.com/publications/16628653>)

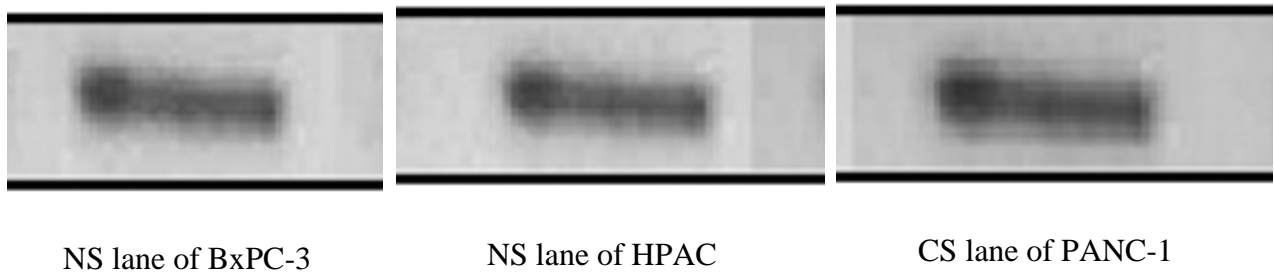
Figures can be seen side by side here:
<http://i.imgur.com/OeiHlr3.png>

71. There are many comparisons being drawn by these comments, so I will describe my analysis of just a few of them. The first paragraph invites comparison between various portions of Figure 1D:



72. The comment first asks for a comparison of (1) the NS lane of BxPC-3, (2) the NS lane of HPAC, and (3) the CS lane of PANC-1. The comment next notes the vertical line and darker background between the fourth and fifth lanes (between the NS lane of HPAC and the CS lane of PANC-1).

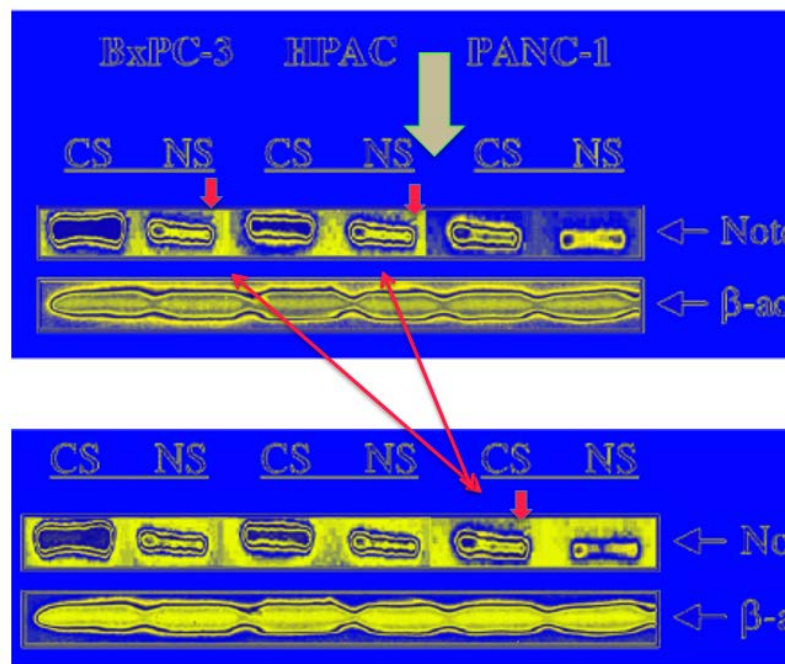
73. Based on a visual comparison, alone, the image does in fact show unexpected and multiple points of similarity between these bands relative to the respective shape, the orientation (rising to the left), and the asymmetrical distribution of band density (i.e., intensity) at the left, at the middle, and at right end of each band. The full Journal image also shows a sharp shift in background intensity between the NS lane of HPAC and the CS lane of PANC-1. Here are just the relevant bands, excerpted from the figures and magnified:



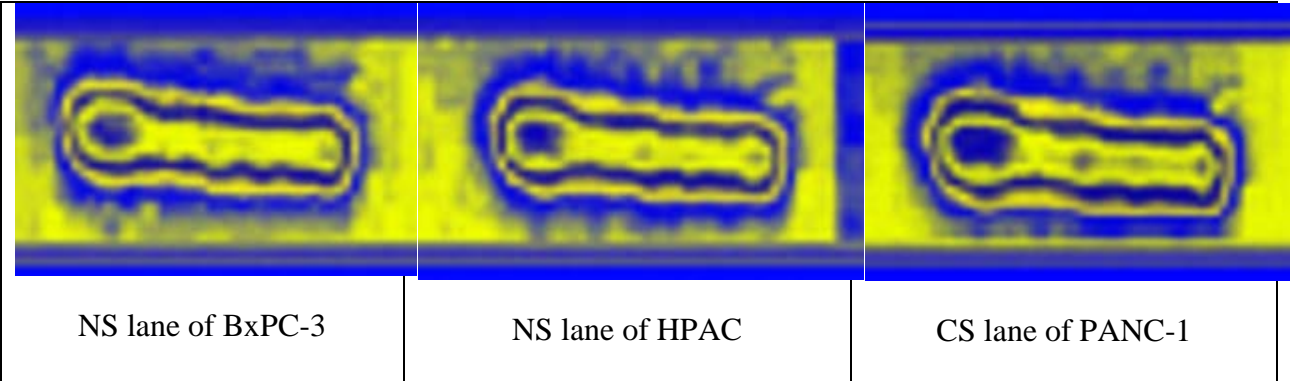
74. The overall similarity between the bands shown in the excerpts is slightly easier to see if their magnification is reduced. However more information can be revealed by examining the features of the bands from the Journal image, as illustrated next.

75. I extracted and copied the image from the enlarged version on the journal's html website source. False-color enhancement of the bands in Figure 1D showed additional features that confirm the similarities and the shift in background intensity. In the false-color images below, the top image shows a color enhancement, which reveals additional similarities between the NS lane of BxPC-3 and the NS lane of HPAC. It also reveals the clear and sharp shift in background intensity that occurs just before the 5th band, consistent with selective photo-editing in the row, which is absent in the associated loading control row (the second row). (A less distinctive, vertical line in the background occurs after the 2nd band.). Moreover, the features of the irregular margins of the 2nd, 4th, and 5th bands show multiple points of coincidence in the

patterns of intensity (noted by the red arrows) in the top illustration below (which consists of two different visualizations of the same image panel).

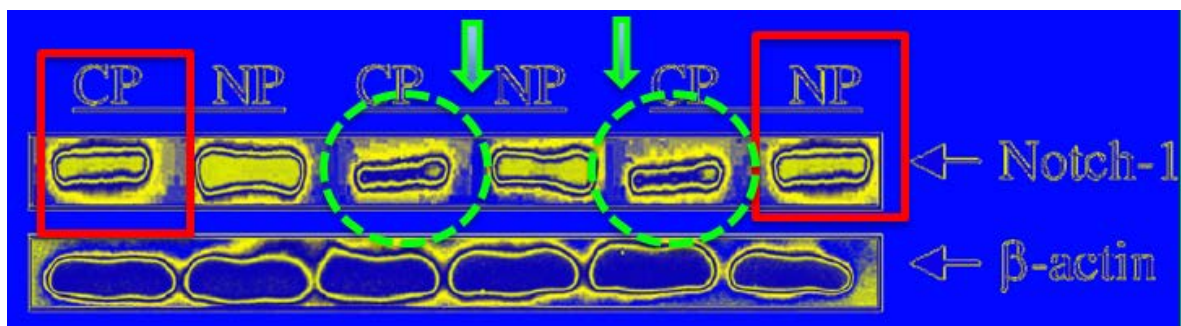


76. The color reversals at the margins of the 5th band are accounted for by the effect in the visualization method of the selective lightening of the 5th and 6th band data. The lower of the two panels shows brightening the same image above by 15 levels does not change the pattern at the band's margin, and now the 5th band also resembles the 2nd and 4th. Thus, evidence shows the similarity of the 2nd, 4th, and 5th in the top Notch-1 row. Here are those three bands, extracted from the false-color image above (with the final band lightened by 15 levels):

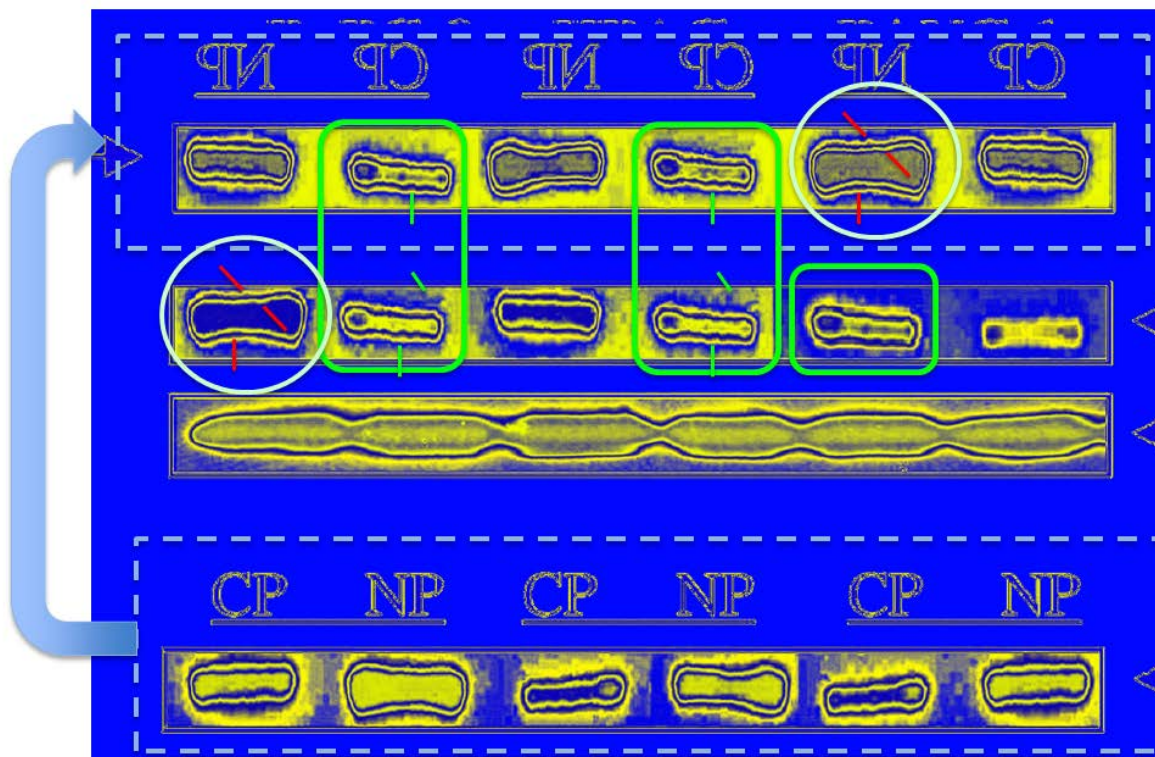


77. On the basis of this analysis and image enhancement of Figure 1D, there is evidence that strongly supports the conclusion that the image is not authentic.

78. The second paragraph of the comment on Figure 1D invites comparison between bands in the lower Notch-1 panel: the CP band of HPAC with the CP band of PANC-1 (the red boxes below); and the CP band of BxPC3 with the NP band of PANC-1 (the green dotted circles below). For the sake of brevity, I will include the false-color image I produced to examine these comparisons without as much explanation. Suffice it to say that there is strong evidence to conclude that the bands identified are not authentic



79. The same is true of the third paragraph of the comment, which invites comparison of two sets of bands once the entire lower Notch-1 panel is flipped: the NP band of BxPC-3 in the lower Notch-1 panel with the CS band of BxPC-3 in the upper Notch-1 panel (circles below); and the CP bands of HPAC and PANC-1 in the lower Notch-1 panel with the NS bands of BxPC-3 and HPAC in the upper Notch-1 panel (green boxes below). Again, for the sake of brevity, the false-color image I created to analyze these bands is produced below, without the same detailed explanations I provided above. Note that the top row in the image has been flipped horizontally (from the row on the very bottom) and lightened to match the background of the other row. The small annotations in the image below show a few of the similar features that led to my conclusion that there is strong reason to believe that the image is not authentic.



80. I will not fully document my analysis here of the remaining paragraphs of the comments in this second example, but in each case, I reached a similar conclusion, that the figures analyzed showed strong evidence to question their authenticity.

81. **Third example.** I will provide one final brief example of my analysis. This third and final example concerns images in the following paper published in 2011: *Inactivation of AR/TMPRSS2-ERG/Wnt Signaling Networks Attenuates the Aggressive Behavior of Prostate Cancer Cells*, Yiwei Li, Dejuan Kong, Zhiwei Wang, Aamir Ahmad, Bin Bao, Subhash Padhye, and Fazlul H. Sarkar.

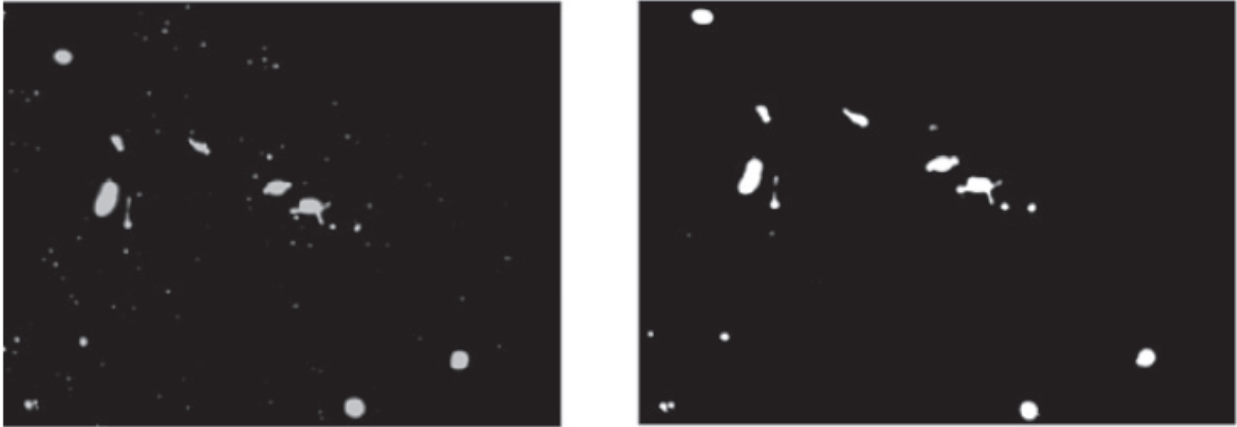
82. One of the several comments that PubPeer commenters made on the article, as provided to me by PubPeer's counsel, was as follows:

Figure 3A

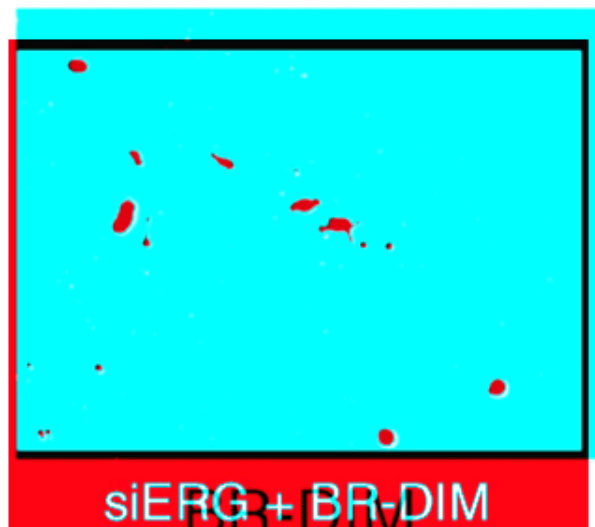
Image of LNCaP, BR-DIM is identical to image of VCaP, siERG + BR-DIM.

Same image for two different cell types and conditions.

83. The images in question, which are below, depict the results of a fluorescence experiment conducted on a population of cells.



84. The shapes, locations, patterns, and intensity of fluorescence emanating from a population of single cells should be fully independent of another population, yet in this case there are points of agreement for at least 13 separate features. I verified this through use of the overlay tool discussed above (Points 38-45), which produced the image below. Given the multiple sources of expected biologic variation, the evidence in support of the conclusion that the images are not authentic is exceptionally strong.



Conclusion.

85. The examples provided above are just a few of the analyses I conducted in examining the 28 separate issues involving ~44 images excerpted from data reported in 25 separately Published Figures (e.g., Figures 6A, 6B, and 6C are taken to be three figures, because they purport to be results of three experiments). With respect to every image or set of images that I examined, I concluded that there was strong evidence to believe that the images at issue were not authentic or contained other irregularities. Although not reproduced above, I would be happy to submit documentation of the balance of my analyses.

86. PubPeer's counsel did not ask me to determine whether the fact that the images I examined are not authentic is evidence of research misconduct by someone involved in the preparation of the papers. To make such a determination one would need direct access to the original data, and a fact-finding process that would require a fuller review by the institution. Had I been presented with these images while still at ORI, I would have recommended that ORI refer the images to the host institution where the research was conducted for such an investigation. Based on my experience at ORI, and given the demonstrable credibility of the numerous issues identified by PubPeer, I believe it very likely that ORI would have made such a referral in this case.

Resources

87. Below is a list of the PubPeer comments provided to me by PubPeer's counsel, along with the names of the eight papers associated with those comments. In all, the comments identified 25 images or sets of images that I examined. A number of the comments came in the form of images.

88. Comments on:

- a. *Down-regulation of Notch-1 contributes to cell growth inhibition and apoptosis in pancreatic cancer cells*, Zhiwei Wang, Yuxiang Zhang, Yiwei Li, Sanjeev Banerjee, Joshua Liao, and Fazlul H. Sarkar.
- b. *Notch-1 Down-Regulation by Curcumin Is Associated with the Inhibition of Cell Growth and the Induction of Apoptosis in Pancreatic Cancer Cells*, Zhiwei Wang, Yuxiang Zhang, Sanjeev Banerjee, Yiwei Li, Fazlul H. Sarkar.

Figure 1D

UPPER Notch-1 panel: please compare NS of BxPC3 (lane 2 from left) with NS of HPAC (lane 4 from left) and CS of PANC-1 (lane 5 from left). Note also the vertical line and darker background on the left side of the CS band of PANC-1.

LOWER Notch-1 panel: please compare CP of HPAC (lane 3 from left) with CP of PANC-1 (lane 5 from left). Also compare the CP band of BxPC3 (lane 1 from left) with the NP band of PANC-1 (lane 6 from left).

Now, please FLIP HORIZONTALLY the entire LOWER Notch-1 band. Now compare the NP band of BxPC3 in the lower Notch1 panel (lane 2 from left in the original) with the CS of BxPC3 in the upper Notch-1 panel (first lane from left). Also compare the CP bands of HPAC and PANC-1 in the lower Notch-1 panel with the NS bands of BxPC3 and HPAC in the upper Notch-1 panel.

Figure 5

Cyclin D1 Panel: please compare the shape and position of the CS band of HPAC with the CS band of PANC-1 in the Cyclin D1 panel (upper).

CDK2 Panel: please note the vertical line between the NS band of HPAC and CS band of PANC-1. Please note the box around the NS band of BxPC3 (magnify).

Figure 6A, B and C

Please compare the Rb bands in the three panels (A, B, and C). Compare the BxPC3 and HPAC bands in 6A and 6B, magnify

and see the shapes and background, especially the small specks in the upper right corner of the second band (from left). Now, please FLIP HORIZONTALLY the RB bands in PANC-1 (panel C) and compare with the two other bands (BxPC3 and HPAC in panes A and B). Then, note the small specks in the upper right corner of the second band (from left).

Figure 7E and Figure 8D

Please compare the two Rb bands. But please increase the width of the Rb bands in Figure 8 and compare. Better seen in PowerPoint, magnify.

Fig. 8A in this paper is identical to Fig. 5A in Cancer, 2006 Jun 1;106(11):2503-13; (<https://pubpeer.com/publications/16628653>)
Figures can be seen side by side here:
<http://i.imgur.com/OeiHlr3.png>

89. Comments on:

- a. *Inactivation of AR/TMPRSS2-ERG/Wnt Signaling Networks Attenuates the Aggressive Behavior of Prostate Cancer Cells*, Yiwei Li, Dejuan Kong, Zhiwei Wang, Aamir Ahmad, Bin Bao, Subhash Padhye, and Fazlul H. Sarkar.

Figure 3A

Image of LNCaP, BR-DIM is identical to image of VCaP, siERG + BR-DIM. Same image for two different cell types and conditions.

Figure 6.

<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3167947/figure/F6/>

PSA panel. Vertical changes in background between lanes 1 and 2, 3 and 4, and between lanes 5 and 6.

No vertical chnages in background in the other 4 panels.

Comparison between spliced and unspliced panels is problematic.

Check this out: same bands for different time conditions
<http://i.imgur.com/4qJBeS7.png>
<http://i.imgur.com/UaeqmWb.png>

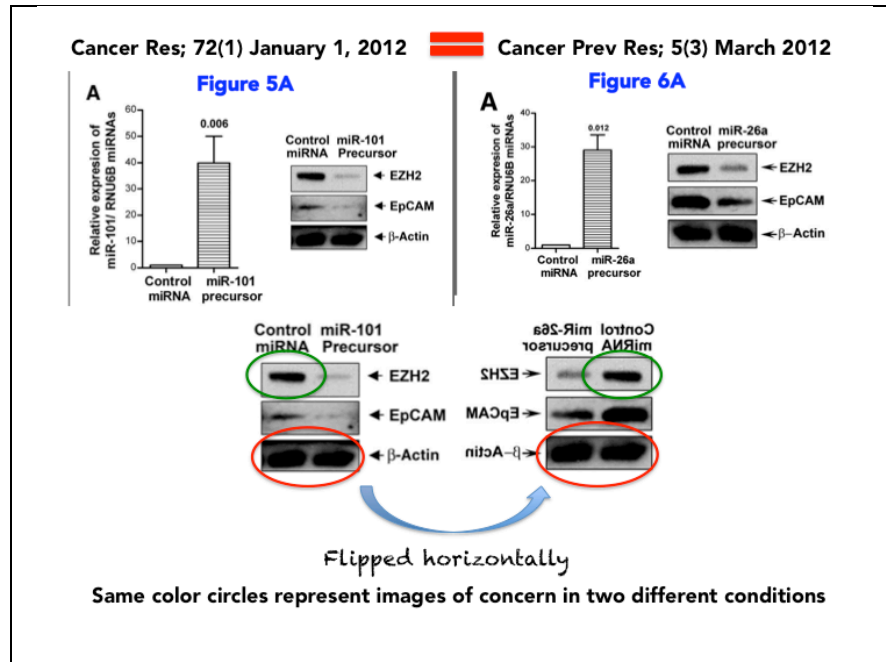
90. Comment on:

- a. *Activated K-Ras and INK4a/Arf Deficiency Promote Aggressiveness of Pancreatic Cancer by Induction of EMT Consistent With Cancer Stem Cell Phenotype*, ZHIWEI WANG, SHADAN ALI, SANJEEV BANERJEE, BIN BAO, YIWEI LI, ASFAR S. AZMI, MURRAY KORC, and FAZLUL H. SARKAR.

The EZH2 band in Figure 4B is the same band for E-Cadherin in Figure 4C, just flipped over 180 degrees.

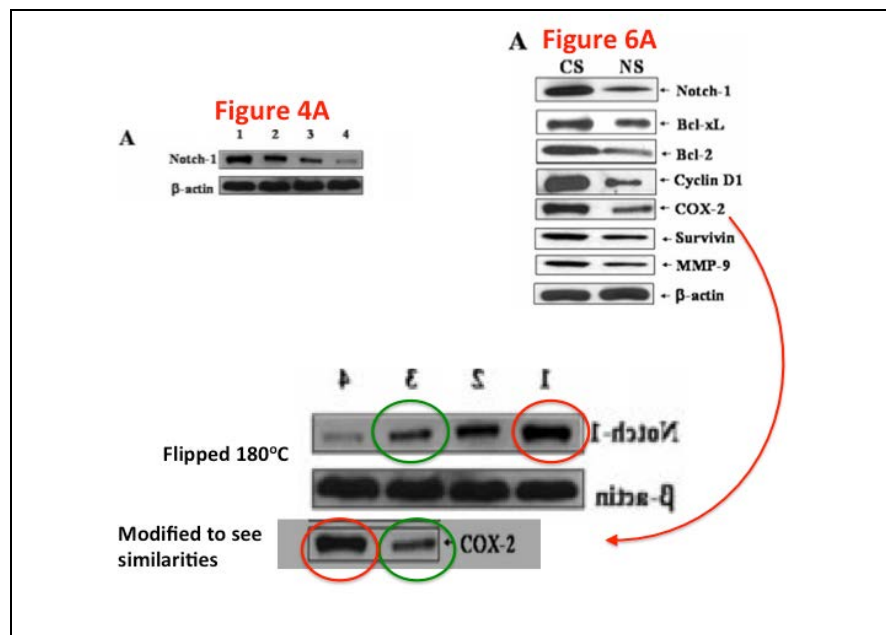
91. Comment on:

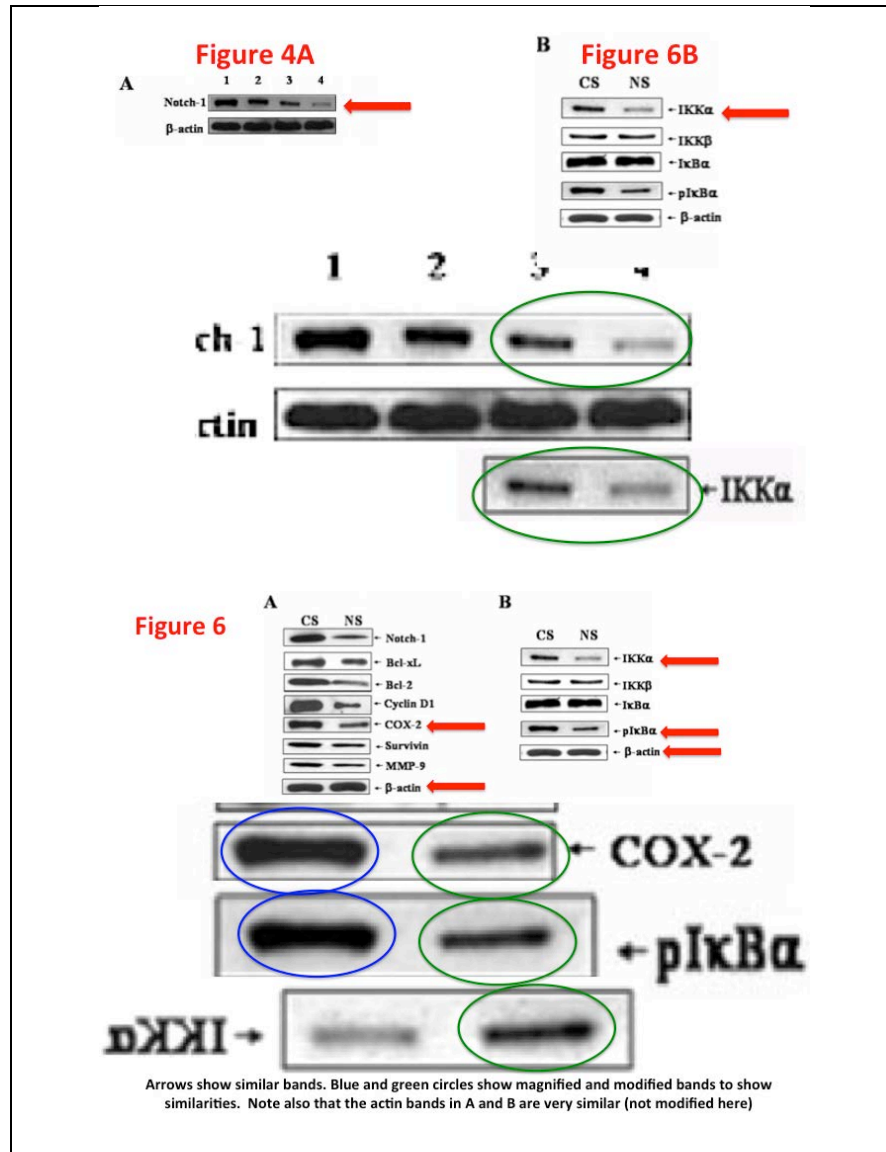
- a. *Metformin Inhibits Cell Proliferation, Migration and Invasion by Attenuating CSC Function Mediated by Deregulating miRNAs in Pancreatic Cancer Cells*, Bin Bao, Zhiwei Wang, Shadan Ali, Aamir Ahmad, Asfar S. Azmi, Sanila H. Sarkar, Sanjeev Banerjee, Dejuan Kong, Yiwei Li, Shivam Thakur, and Fazlul H. Sarkar.
- b. *Curcumin Analogue CDF Inhibits Pancreatic Tumor Growth by Switching on Suppressor microRNAs and Attenuating EZH2 Expression*, Bin Bao, Shadan Ali, Sanjeev Banerjee, Zhiwei Wang, Farah Logna, Asfar S. Azmi, Dejuan Kong, Aamir Ahmad, Yiwei Li, Subhash Padhye, and Fazlul H. Sarkar.



92. Comments on:

- a. *Inhibition of nuclear factor κ B activity by genistein is mediated via Notch-1 signaling pathway in pancreatic cancer cells*, Zhiwei Wang, Yuxiang Zhang, Sanjeev Banerjee, Yiwei Li and Fazlul H. Sarkar.



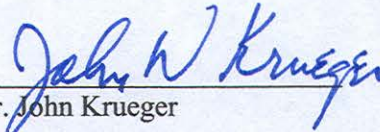


93. Comments on:

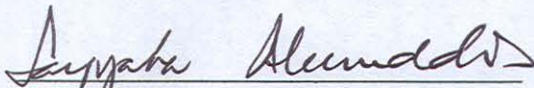
- a. *Molecular Evidence for Increased Antitumor Activity of Gemcitabine by Genistein In vitro and In vivo Using an Orthotopic Model of Pancreatic Cancer*, Sanjeev Banerjee, Yuxiang Zhang, Shadan Ali, Mohammad Bhuiyan, Zhiwei Wang, Paul J. Chiao, Philip A. Philip, James Abbruzzese, and Fazlul H. Sarkar.

Compare Fig. 3B and Fig. 3D

When Colo357 lane for 0 and 25 in 3B is flipped it looks similar to the control and genistein in Fig. 3D for Colo357.


Dr. John Krueger

Signed and sworn before me this 9 day
of December, 2014


Notary Public



TAYYABA ALEEMUDDIN
NOTARY PUBLIC STATE OF MARYLAND
My Commission Expires November 10, 2015

Exhibit A

November 21, 2014

JOHN W. KRUEGER, Ph.D

Expertise: Forensic Examination of Questioned Images in Science

General background:

My direct expertise stems from 20 years of relevant Federal work, my second career starting as one of the original Investigator/Scientists in the Division of Research Investigations (or later the Division of Investigative Oversight), Office of Research Integrity (1993-2013). In this position, I was responsible for oversight of investigations into allegations of falsification of research. This task involved a heavy commitment to forensic assessment of the evidence, either for the allegations (sometimes made anonymously) for referral to institutions, or in the evaluation of the resultant institutional finding. This was one of the more interesting jobs in science, as it providing many opportunities. At ORI, I:

- Pioneered and developed *de novo* the image processing methods for forensic examinations, including ORI's Forensic Tools, that are available on the ORI website (see links below). These tools have been provided and used world-wide, and they have been subject of articles both here and on the internet.
- Developed the interpretations of the results, and advised and supported Journal editors in these matters;
- Trained numerous others in these methods (including my ORI colleagues and numerous institutional officials and faculty members) who were doing the investigations, as well as journal production editors doing image screening;
- Was heavily involved in education of the community about these new forensic methods and their interpretation. (See links to articles, material about ORI's forensic tools, and list of presentations, below; any item is available upon specific request);
- Established the Image Forensics Lectures for Institutional Officials at ORI's RIO BootCamp program (BootCamps I-VII);
- Established and successfully maintained the Macintosh Computer Forensics and software support in ORI (despite OASH recalcitrance due to federal preferences for the PC platform). As part of this responsibility I also laid out the group Forensics lab ("Harvey's room" at ORI);
- My experience includes working closely with lawyers in defending ORI position regarding appeals of PHS findings the HHS Departmental Appeals Board.

Just as important as the ORI experiences working actual cases, an expertise in the judicious interpretation of the results of testing questioned images in science. This skill

November 21, 2014

stems from my first career, which culminated in running my own laboratory as an independent, NIH-supported bench researcher and senior faculty member at the Albert Einstein College of Medicine (1975-1993). Prior to ORI I obtained a Ph.D. in Biomedical Engineering from Iowa State University ('71); I then trained at Imperial College, London ('72), was a *locum* lecturer at the Royal Free Hospital School of Medicine, and then a postdoctoral fellow at the center for Bioengineering University of Washington in Seattle ('72-'75). At AECOM, I:

- Was peer reviewer for multiple papers in cardiac cell physiology, and served as an expert reviewer for NIH site visits for four program projects.
- Taught medical undergraduates, graduate students and postdoctoral fellows, and ten NYAS summer research interns (high-schoolers).
- Was an established Fellow of the New York heart Association and the Wunsch Fellow in "Biophysical Engineering."
- My laboratory at pioneered the laser diffraction methods for studying contraction the subcellular level in heart muscle, first reported the contraction of the isolated heart cell. The latter methods became a common tool in pharmaceutical industry.
- With an MD-Ph.D student, now director of Cardiology at University of Pittsburgh, the laboratory pioneered successful application of a new method to study excitation-contraction coupling in the single heart cell, that has formed the platform for more advanced techniques by others.
- I also generated two patents on micromanipulators and hydraulic control (US Patent Office #4,946,329 and #5,165,297) that received commercial attention.

(A complete pre-ORI academic CV and list of publications is available upon request).

Relevant Formal Training in Federal Law Enforcement, Investigations, and Image Processing:

- Introduction to Criminal Investigations, Federal Law Enforcement Training Center (FLETC), Glynco, Ga. 1994.
- Computer Evidence Analysis Training Program, Financial Fraud Institute, Federal Law Enforcement Training Center (FLETC), Glynco, Ga. 1994.
- Image Processing on the Macintosh, Division of Computer Research and Technology, Benos Trus, NIH DCRT, 1994.
- Advanced Interviewing Techniques, Federal Law Enforcement Training Center (FLETC), Glynco, Ga. 1995.
- Forensic Psychiatry and Questioned Documents Examination, George Washington University Continuing Education Program (taught at ORI), 1996.
- Short Course on The Detection of Deception (Reid Technique), by Joe Buckley, provided at ORI. (~1998)
- Introduction to the Image Processing Toolkit, John Russ. Image Processing Short Course, North Carolina State University, Raleigh, NC. May 1998.

Papers for ORI

- John Krueger, “Images as Evidence: Forensic Examinations of Scientific Images,” pp. 261-268 in “Investigating Research Integrity, Proceedings of the First ORI Research Conference on Research Integrity,” NH Steneck and MD Scheetz, (Eds) DHHS/ORI Publication, 2000.
- John Krueger, “Forensic Examination of Questioned Scientific Images,” in *Accountability in Research* 9: 105-125, 2002. This is the first description of ORI’s methods and the use of image processing to examine questioned images in science. (Later I was invited to tour the FBI Image processing lab, where I learned the FBI provided this paper to new trainees in the FBI image processing lab.)
- James E. Mosimann, John E. Dahlberg, Nancy M. Davidian, and John W. Krueger, “Terminal Digits and the Examination of Questioned Data,” in *Accountability in Research* 9: 75-92, 2002.

ORI Newsletters on Image Processing, and Issues of Image Falsification, Corrections

<http://ori.hhs.gov/newsletters>

- [Krueger, John W] Image Processing Useful in Misconduct Investigations.” *ORI Newsletter* 3(2): 6, March 1995. [This is apparently the suggestion for this approach in the analysis of questioned images in science.) It was soon uploaded by another on the NIH-Image Bulletin board on March 31, 1995.]
- John Krueger, “Confronting Manipulations of Digital Images in Science,” *ORI Newsletter* 13(3): 8-9, June 2005. [This paper reported the results of tracking the increase in ORI’s image falsification cases, and cited website for the newly created Forensic Tools.]
- John W. Krueger, “Journal Audits of Image Manipulation,” *ORI Newsletter* 17(1): 2-3, December 2008.
- John Krueger, “Incidences of ORI cases involving falsified images.” *Office of Research Integrity Newsletter* 17(4): pp. 2-3, September 2009.
- Sandra Titus, Ph.D., John Krueger, Ph.D., and Peter Abbrecht, MD, Ph.D., “Promoting Integrity in Clinical Research,” *ORI Newsletter* 19(4): 1-3, September 2011.
- John Krueger, “Further Correcting the Literature: PubMed “Comments” Link Publications to PHS Research Misconduct Findings,” *ORI Newsletter* 19(4): 4-8, September 2011.
- John Krueger, Ph.D., “What do Retractions Tell Us?” *ORI Newsletter* 21(1): 1-6, December 2012. (page 2 missing?)

(ORI Story on My Retirement: ORI Newsletter 21(3): 3, June 2013)

November 21, 2014

ORI Related Video Interviews/Demonstrations:

- Image Processing Case Demonstration filmed for Japanese Television Program, shown on NHK Tokyo TV, February 8, 2006.
- Three “Expert Interviews” for university of Texas video training (present on ORI website)
- Image Processing Case Demonstration filmed for one hour BBC television program on scientific misconduct, “Betrayers of the Truth,” 1994.

Components of ORI Website and RCR training:

- Handling Misconduct: - Technical Assistance, Image Processing in Research Misconduct cases, ORI website
http://ori.dhhs.gov/misconduct/Tips_ImageProcessing.shtml.
- Initiated and Created of ORI’s Forensic Tools, i.e, Forensic Droplets and Actions, starting in 2005 <http://ori.hhs.gov/actions>, and updated in July 2012
<http://ori.hhs.gov/advanced-forensic-actions> including explanatory Read Me Files on Image searching and interpretation. These are ORI forensic tools for the Examination of Scientific Images on ORI Websites <http://ori.hhs.gov/forensic-tools>
- RCR Educational Resource Material:
http://ori.dhhs.gov/education/products/rcr_misconduct.shtml. Links for three web interviews, as ORI expert for Image Analysis: University of Texas Research Misconduct Training Program, Melissa Proll, Ph.D, located at http://www.uth.tms.edu/orc/training/Research_Misconduct/index.htm
- Initiated and developed the ORI Forensic Video Project, a novel step by step video demonstration of good forensic setup and analysis technic with Photoshop, which was produced professionally and completed through the first phase that involved methods that were provided in support of ORI cases.
<http://www.cmc2.tv/forensic/> (This website was never publicly released, however, since it lacked support for public release and the content is still relevant, but the demonstration has since become dated by the version of Photoshop.)

Supporting Journals and the Scientific Community

- Organizer of workshop at ORI to hear perspectives of selected experts in computer image processing, NIH researchers, and journal editors: “Image Manipulation Workshop: Guidelines and Tools,” ORI, January 25, 2005. External participants including Drs. Hani Farid (Dartmouth University), Mike Rossner (Managing Editor, JCB, Kenneth Yamada (NIH) and others.

November 21, 2014

- Provided innumerable confidential consultations to Journal editors about specific cases.
- Provided ORI Forensic Tools per request to many Journals (including Nature, Science, FASEB, American Microbiological Society, JBC) and to many foreign institutions including the Pasteur Institute (Paris), one of the Max Plank Institutes, and a Swedish University. I also have provided the tools upon request to scientists teaching RCR, to a defense lawyer, and even (I suspected) to a potential respondent. The tools have been used in reporting allegations to ORI, and by at least one reporter in advancing her story.

Intern Training:

Successfully applied/obtained HHS funding for Government Intern Forensic Training; Trained Jennifer Urbanowski (graduate student from Forensic Science program, George Washington University). Spring and summer, 2004.

Presentations for ORI: 2013 – 1994 (reverse chronological order)

(PowerPoints of specific talks available upon request)

(Separate sessions color-keyed for Image Forensic Training:

for **Journal Production Editors** are **hi-lited in Green**;

for **Institutional (university) Research Integrity Officials** are **hi-lited in Red**;

for **NIH Research Administrator** are **hi-lited in Blue**.

1. “Retractions, problem Images, . . . and the “Future?” AAAS Washington, DC April 15, 2013 (Assembled Editors in Washington DC, and via Web, European editors in Cambridge, Paris, and South America)
2. **“Some Forensic of Scientific Images” – Technical Session for Art Editors, AAAS, April 15, 2013 (Assembled Editors in Washington DC, and via Web, European editors in Cambridge, Paris, and South America)**
3. John W. Krueger, “Image Forensics Issues in ‘Research Misconduct’ Cases.” Joint AAAS-ABA Committee, National Conference of Lawyers and Scientist, AAAS, Washington, DC. March 14, 2013.
4. “Retractions, Problem Images, and Their Detection,” Discussion/Demonstration for the American Society for Nutrition and the Publication Editors, Federation of American Societies for Experimental Biology (FASEB), Bethesda, MD, December 14, 2012.
5. “Confronting Integrity Issues in Publishing,” American Society for Biochemistry and Molecular Biology (ASBMB) Publications Committee (Web Meeting), October 23, 2012.
6. “Image Integrity in Publishing Scientific Data,” The Borden Institute, Fort Detrick, MD, 9-11am, September 7, 2012.

November 21, 2014

7. "Principles in Assessing Integrity in Scientific Publishing," The Borden Institute, Fort Detrick, MD, 9-11am, September 7, 2012.
8. "Public Awareness and the Detection of Research Misconduct," Nature Publishing Group, New York, NY, July 23, 2012.
9. "Principles in Assessing Image Allegations," Training Demonstration Session, Nature Publishing Group, New York, NY, July 23, 2012.
10. Introductory Comments and slides for "Setting the Research Record Straight," Presentation and Panel Member, Science Online New York City (SoNYC), Rockefeller University, New York, NY, March 20, 2012, <http://sonyc9-eorg.eventbrite.com/> and <http://www.livestream.com/sonyc> (A video of this talk and panel discussion was available.)
11. "Research Misconduct – Not 'If' but 'When,'" ORI Presentation to NIH ESA Seminar Series, December 16, 2011.
12. "De-Authenticate" What's wrong and Why? PowerPoint Training Puzzle examples of closed ORI cases provided to NIH ESA Seminar participants, December 16, 2011.
13. "Image Integrity in Scientific Publishing," Annual Meeting, Council of Science Editors Annual Meeting, Baltimore, MD; May 1, 2011.
14. "Research Misconduct – It Happens," ORI Presentation to NIH ESA Seminar Series, Bethesda, MD; 1-2:30pm March 11, 2011.
15. "Wrestling with Research Misconduct," ORI Presentation to NIH Extramural Scientist Administrator (ESA) Seminar Series, Bethesda, MD; 1-2:30 pm, January 28, 2011.
16. Discussant; *Panel for Session on Research Integrity, Government University-Industry Round Table (GUIRR)*; National Academy of Sciences; July 27, 2010
17. "Image Manipulation and Analysis" **Videocast**; *NIH Extramural Staff Training Seminar; Handling Allegations of Research Misconduct*; Natcher Bldg; NIH; Rockville, MD; July 13, 2010 http://odoerdb2.od.nih.gov/oer/training/esa/esa_training_20100713.htm
18. "Investigating Research Misconduct -Tools-of-the Trade" *3rd Biennial IdeaA Conference*; 2 hour presentation, Workshop Session 3, *NISBRE, NCRR*; Bethesda, MD; June 18, 2010 (NIH sponsored meeting for career skills of junior faculty members)
19. "Digital Manipulation of Images in Science (Session 1-Overview)" *American Society for Microbiology*; Washington, DC; April 20, 2010
20. "Digital Manipulation of Images in Science (Session II- Technical Aspects and Demonstration)" *American Society for Microbiology*; Washington, DC; April 20, 2010
21. "ORI 'Forensics': Examining Questioned Images." Boot Camp VII, University of Oregon, Eugene, Oregon, October 13, 2009.
22. "The Vogel Case: What are the Allegations? [Handling] Questioned Images." Boot Camp VII, University of Oregon, Eugene, Oregon, October 13, 2009.
23. "Evidence in the Oversight of Investigations," Boot Camp VII, University of Oregon, Eugene, Oregon, October 13, 2009.
24. "ORI 'Forensics': Examining Questioned Images." RIO Boot Camp VI, Northwestern University, Chicago, Illinois, June 9, 2009.

November 21, 2014

25. "Evidence in the Oversight of Investigations," RIO Boot Camp VI, Northwestern University, Chicago, Illinois, June 9, 2009.
26. "Detection of Image Manipulation – How-to's and What-if's," American Physiological Society, at FASEB, Bethesda, Maryland, May 28, 2009.
27. "Image Demonstration and Points," American Physiological Society, Production Editors, at FASEB, Bethesda, Maryland, 12-2pm, May 28, 2009.
28. "ORI's Forensics: Questioned Images in Science," RIO Boot Camp V, Tulane University New Orleans, LA, November 18, 2008
29. "How Evidence Informs the Investigation." RIO Boot Camp V, Tulane University New Orleans, LA, November 19, 2008
30. "Falsification of Images in Science," Workshop on "Investigating Research Misconduct," Second Biennial NISBRE, NIH-NCRR Meeting, Wardham Park Marriott, Washington, DC, August 8, 2008. (NIH sponsored meeting to promote career skills of junior faculty members)
31. "Falsified Images in Science," Discussion Group in Research Misconduct, Public Service, Public Trust, Uniformed Services University in the Health Sciences, Bethesda, MD, July 23, 2008.
32. "How Evidence Informs the Investigation." RIO Boot Camp IV, University of Washington, Seattle, WA, June 1-4, 2008.
33. "ORI's Forensics: Questioned Images in Science," RIO Boot Camp IV, University of Washington, Seattle, WA, June 4, 2008.
34. "Image Manipulation/Falsification in Science – Detection and Choices," Emerging Trends in Scholarly Publishing, Allen Press Seminar, National Press Club, Washington, DC, April 17, 2008.
35. "ORI's Forensic Examination of Questioned Images in Science." RIO Boot Camp III, Poynter Center, Indiana University, IN, April 2, 2008.
36. "Analysis of the Case Images." RIO Boot Camp III, Poynter Center, Indiana University, IN, April 3, 2008.
37. "ORI Forensics" Examination of Questioned Images in Science. RIO Boot Camp II, Johns Hopkins University, Baltimore, MD, November 4, 2007.
38. "Vogel – Case Boot Camp Analysis." RIO Boot Camp II, Johns Hopkins University, Baltimore, MD, November 7, 2007.
39. "Detection and Interpretation of Manipulated Images in Science," Plenary Session, Annual Meeting of the Council of Science Editors, Austin, TX, May 20, 2007.
40. "ORI 'Methods': Examination of Questioned Images in Science," ORI/Harvard Medical School/Harvard School of Public Health, Harvard Teaching Hospitals Conference "Data Fabrication and Falsification: How to Avoid Detect, Evaluate and Report," Boston, MA, March 29, 2007.
41. [Copy of presentation above provided per request to Publication Director, ASBMB Publications, April 5, 2007.]
42. "ORI Forensics" Examination of Questioned Images in Science. RIO Boot Camp I, University of Michigan, Ann Arbor, MI. May 4, 2007.
43. "Vogel – Case Boot Camp Analysis." RIO Boot Camp I, University of Michigan, Ann Arbor, MI. May 4, 2007.

November 21, 2014

44. "Detection and Interpretation of Falsified Images in Science, Nature Publishing Group, New York City, April 25, 2007.
45. "Image Forensics," Demonstration and Training session: Nature Publishing Group, New York City, April 25, 2007.
46. "Confronting Digital Manipulation of Images (and Research Misconduct)," Discussion Nature Publishing Group, NYC, March 22, 2006.
47. "Image Manipulation in Science," presentation and working discussion on image screening for senior staff and Dr. Donald Kennedy, AAAS headquarters, Washington, DC. December 2005. (Science publicly announced that it would prescreen selected articles on December 22, 2005.)
48. (On site RRTA) 3 hour presentation to Institutional Investigative Committee on ORI Image Analysis, Milwaukee, WI., Thursday, July 21, 2005.
49. "Digital Manipulation of Images in Research and Scientific Misconduct," Drake University, Des Moines, IO, March 3, 2005.
50. "Digital Manipulation of Images in Research and Scientific Misconduct," Iowa State University, Ames, IO, March 4, 2005.
51. "Where Responsible Conduct of Research Meets Scientific Misconduct," Iowa Health, Des Moines, IO, March 4, 2005, 2005.
52. "Image Manipulation Workshop: Guidelines and Tools," ORI Meeting with Invited Experts, January 25, 2005
53. "Falsification of Images in Science," (CME Credit) Medical University of South Carolina, Charleston, SC, September 30, 2003.
54. "Color Tagging for Interpreting Overlap in Questioned Gray Scale Images," talk and poster at the 2002 ORI Research Conference on Research Integrity, Bolger Center, Potomac, MD, November 17, 2002.
55. "Images as 'Evidence' - Recognizing and Investigating Scientific Misconduct," Medical College of Wisconsin, Milwaukee, WI, May 1, 2002.
56. "Recognizing and Investigating Scientific Misconduct," National Council of University Research Administrators' Region IV Meeting, Madison, WI, April 30, 2002.
57. "Case Study: Uncooperative Respondent and Working with Experts - Scientific Preparation for Departmental Appeals Board (DAB) Hearing," ORI Advanced Investigative Techniques for Research Misconduct, Lister Hill Center, NLM, Bethesda, MD, March 20, 2002.
58. "ORI Image Analyses - General Approach and Methods," ORI Advanced Investigative Techniques for Research Misconduct, Lister Hill Center, NLM, Bethesda, MD, March 21, 2002.
59. "Demonstrations of ORI Computer Analyses - Image Processing," walk-around demonstration table at the ORI Advanced Investigative Techniques for Research Misconduct, Lister Hill Center, Bethesda, MD, March 21, 2002.
60. "Recognizing and Reporting Scientific Misconduct," American Speech Hearing Association/ORI conference on Promoting Research Integrity in Communications Sciences and Disorders and Related Disciplines. May 3-4, 2001, Rockville, MD
61. "Research Misconduct - The [NSF and the] ORI Experience" at a meeting entitled *Research Integrity - Who is Responsible?*, sponsored by University of South Alabama in Mobile, AL, on April 17, 2001.

November 21, 2014

62. Advanced Investigative Techniques for Research Misconduct workshop, sponsored by ORI, Harvard Medical School, and the University of Pittsburgh, September 24- 25, 2001, in Bethesda, MD.
 - a. "ORI Image Analysis - General Approaches and Methods"
 - b. "Comments" on an image case study presentation given by Dr. L. Wittie, SUNY
 - c. Case studies on "Dealing with Uncooperative Respondents,"
 - d. Case studies on working with experts and the Departmental Appeals Board at the ORI.
63. "Recognizing and Reporting Scientific Misconduct" at the conference sponsored by ORI and ASHA on Promoting Research Integrity in Communications Sciences and Disorders and Related Disciplines, held May 3-4, 2001, in Rockville, MD.
64. "Images as Evidence: Forensic Examination of Scientific Images," at the ORI sponsored "Research Conference on Research Integrity," in Bethesda, MD, on November 20, 2000.
65. "Investigative Methods," in Break out Session, AAAS-ORI meeting, "Responding to Allegations of Research Misconduct, Inquiry, Investigation, and Outcomes: A Practicum,": St. Charles, IL June 5, 2000.
66. Break out session on Misconduct/Responsible Conduct of Science, at Federal Funding Opportunities, A Conference for Researchers and Research Administrators," Friday Center, UNC, Chapel Hill, NC, April 11-12, 1996
67. "ORI Investigations and Issues in Scientific Misconduct." Department of Biology, Iona College, New Rochelle, NY, October 16, 1995.
68. "Allegations of Research Misconduct in U.S. Academic Institutions." Bioethics Center, University of Maryland-Baltimore, April 20, 1995.
69. "Myths, Misconduct, and the Office of Research Integrity." William Paterson State College, Paterson, NJ, October 24, 1994
70. Panelist for Discussion on Misconduct in Science, MARC Scholars program, for talks celebrating inauguration of new President, City College of New York, NY, October 8, 1994.
71. "Image Processing in the Forensic Analysis of Figures", ORI Poster at the National Academy of Sciences Convocation of Scientific Misconduct, NAS bldg., Washington DC, June 6-7, 1994.
72. "DRI Extramural Interactions," ORI Poster at the National Academy of Sciences Convocation of Scientific Misconduct, NAS bldg., Washington DC, June 6-7, 1994.
73. "Federal Response to Investigations of Scientific Misconduct," for course Responsible Conduct of Research, Center for Biomedical Ethics, University of Maryland, Baltimore, MD, 4 pm, April 20, 1994.