



European Journal of Histochemistry

SUPPLEMENTARY MATERIAL

DOI: [10.4081/ejh.2023.3812](https://doi.org/10.4081/ejh.2023.3812)

Management of autofluorescence in formaldehyde-fixed myocardium: choosing the right treatment

Zhao Zhang,¹ Hongming Fan,¹ William Richardson,² Bruce Z. Gao,¹ Tong Ye^{1,3}

¹Department of Bioengineering, Clemson University, Clemson, SC

²Department of Chemical Engineering, University of Arkansas, Fayetteville, AR

³Department of Regenerative Medicine and Cell Biology, Medical University of South Carolina, Charleston, SC, USA

Correspondence: Tong Ye, Department of Bioengineering, Clemson University, 68 President Street, MSC 501, Charleston, SC 29425, South Carolina, USA. E-mail: ye7@clemson.edu

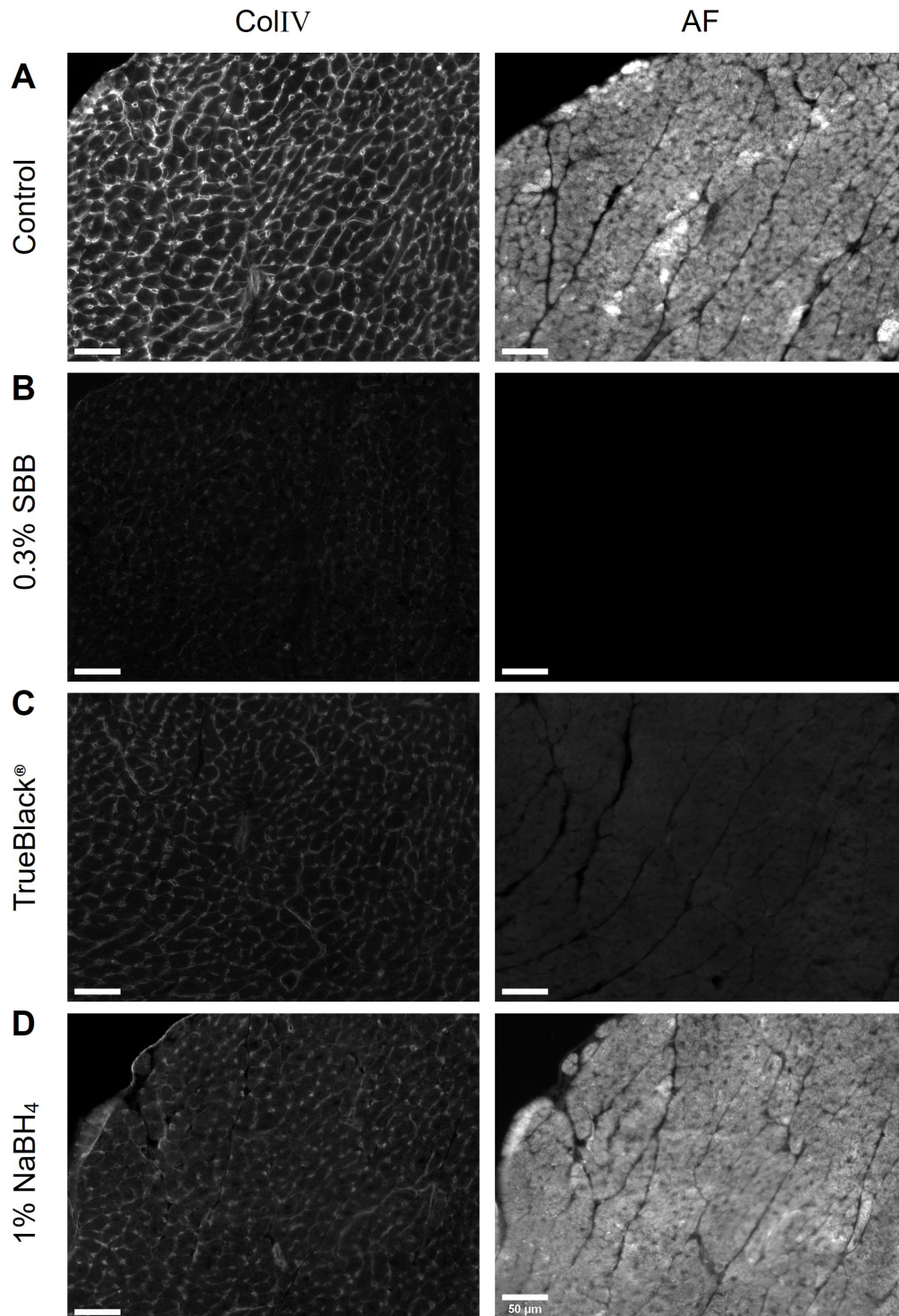
Key words: immunofluorescence; autofluorescence suppression; autofluorescence enhancement; myocardium; formaldehyde.

WIDEFIELD FLUORESCENCE MICROSCOPY

Widefield fluorescence imaging was performed on a Zeiss Axio Imager M2 microscope equipped with a Zeiss AxioCam MRm camera and an EC Plan-Neofluar 20x, NA 0.45 objective. The widefield autofluorescence (AF) images were acquired using the FITC setting, and the widefield immunofluorescence (IF) images were acquired using the Cy5 setting. Samples that underwent the same application protocol were imaged with the same exposure time. Raw images were acquired in 16-bit grayscale. Histogram matching was conducted independently for both IF and AF images of treated samples. Their histograms were adjusted to match the histograms of the images from the corresponding control. Except for histogram matching, no further image processing was applied.

Supplementary Figure 1.

Widefield IF and AF images of (A) control and samples treated with (B) 0.3% SBB, (C) TrueBlack®, and (D) 1% NaBH₄ using the pre-treatment application protocol. Scale bars: 50 μm.



Supplementary Figure 2.

Widefield IF and AF images of (A) control and samples treated with (B) 0.3% SBB, (C) TrueBlack[®], and (D) 1% NaBH₄ using the post-treatment application protocol. Scale bars: 50 μm.

