

Low-Cost, Label-Free Blue Light Cystoscopy through Digital Staining of White Light Cystoscopy Videos

Corresponding Author: Dr Audrey Bowden

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The paper is a proof of concept. Authors studied how to convert standard WL cystoscopy imaging to BL imaging by means of digital computing without the need for instillation or specific equipment. Basically, it is a great idea because advantage of BL cystoscopy would be maintained leaving behind disadvantages. Their software reaches a 80% accuracy respect to the standard technique of BL imaging.

I'm a clinician, my main concerns are:

- 1) How does the 80% accuracy of dsWLC respect so standard BLC translate in terms of increased detection rate respect to standard WL cystoscopy?
- 2) Is it possible to think to a real time conversion with the current technology. . Without it, there is no practical application unfortunately.
- 3) dsWLC overcomes theoretically one of the main problems of BLC. Namely, the chance to move to or from dsWLC to WLC anytime during the procedure. What about photo bleaching?

Reviewer #2

(Remarks to the Author)

The paper is interesting and well written. It is obviously a step forward in diagnosis, but of course not as efficient as it should be on long term. Nevertheless the paper has the merit to be a preliminary work. I think that accuracy is still not extremely satisfying.

The shown pics are very convincing, but it would have been nice to show some pics which were more doubtful in order to evaluate the problems in daily practice.

It would also be nice to say some words about the ground truth, which is a lways as good as the people you chose.

Given the pretty complicated procedure, I am not sure, whether this technique can be easily implemented into daily practice as it is at the monemnt.

Version 1:

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The paper has been improved significantly.

Reviewer #2

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The paper is interesting and well written and sticks to modern concepts.....It is a shame that the relation with histology was not done.

The authors could maybe insist a bit more on Cis and atypical changes in the bladder and their "trouble" with this

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Our responses to each of the reviewer's comments are listed below. The reviewer's original comments are shown in bold. Updated texts are shown in red. Please note that references made in this document are re-numbered compared to how they appear in the revised manuscript.

Reviewer #1 (Remarks to the Author):

The paper is a proof of concept. Authors studied how to convert standard WL cystoscopy imaging to BL imaging by means of digital computing without the need for instillation or specific equipment. Basically, it is a great idea because advantage of BL cystoscopy would be maintained leaving behind disadvantages. Their software reaches a 80% accuracy respect to the standard technique of BL imaging.

Thank you for the comments and for acknowledging the importance of the clinical challenge we are hoping to address in the paper. We appreciate the opportunity to revise our manuscript.

I'm a clinician, my main concerns are:

1) How does the 80% accuracy of dsWLC respect so [sic] standard BLC translate in terms of increased detection rate respect to standard WL cystoscopy?

Because the goal of this proof-of-concept research was to demonstrate the feasibility of digital staining on cystoscopy images, BLC frames were used as the ground truth. No biopsy confirmations were collected in this study; therefore, we were not able to confirm the actual clinical performance of dsWLC compared to WLC. However, published research already shows that the use of BLC allows identification of up to 14% more of cases of Ta and 40% more cases of CIS than WLC alone [1]. Since we achieved our goal of high accuracy in generating digitally stained cystoscopy images from WLC frames that closely resemble BLC frames, it is likely that this translates into an increased detection rate for dsWLC compared to standard WLC to the extent that the ground truth BLC had a higher detection rate.

In our study, we introduced a threshold to quantitatively assess the staining performance on the pixel level, as shown in Figure 7. The 80% accuracy reported in the manuscript was based on the comparison to BLC frames. Ultimately, a full comparison to WLC will need to involve a clinical study to compare dsWLC to the standard of care, likely utilizing biopsy data to calculate accuracy metrics. It is possible and even likely that dsWLC will not only show a sensitivity advantage over WLC due to its deriving from BLC, but also may show a specificity advantage over BLC since it has WLC data as the model input.

In lines 275-277 on page 9 of the revised manuscript, we added the following to the discussion:

“We expect that future efforts to recruit more patients and obtain histological information can lead to increased accurate labeling of CIS tumors, contributing to solving one of the most vexing problems in bladder cancer detection. In future studies, the collection of larger patient datasets and tissue type confirmation from biopsy will enable calculation of true sensitivity and specificity metrics for the dsWLC approach that can be compared against standard white light imaging.”

[1] Fradet Y., Grossman H.B., Gomella L., Lerner S., Cookson M., Albala D., Droller M.J., PC B302/01 Study Group A comparison of hexaminolevulinate fluorescence cystoscopy and white light cystoscopy for the detection of carcinoma in situ in patients with bladder cancer: A phase III, multicenter study. *J. Urol.* 2007;178:68–73; discussion 73. doi: 10.1016/j.juro.2007.03.028.

2) Is it possible to think to a real time conversion with the current technology. Without it, there is no practical application unfortunately.

We appreciate this comment from the reviewer, and we agree that real-time implementation of this technology is crucial to ensure practicality. Yes, we believe that real-time processing is possible. In our current algorithm, there are two main steps for processing: color adaptation and digital staining. The latter digital staining task was performed with the use of a GAN, which others have already shown can perform real-time conversion of images when implemented with suitable graphics processing units [1-4].

The former task of color adaption is currently implemented via optimal transport and is the most time-consuming step of our algorithm. However, a more streamlined version of our algorithm could replace these preprocessing steps with a neural network such as a GAN or CNN to reduce the computational burden [1], which would enable real-time performance. We add this future direction to the revised Discussion section, in lines 241-255 page 9:

“In addition, real-time conversion of WLC into dsWLC is an important consideration to ensure practicality of this approach. To enable clinical translation of the proposed technology, a future task would be to address the most time-consuming step in the pipeline: color adaptation. Several groups have suggested that real-time color adaptation is possible. GANs, SSL (self-supervised learning) models, and CNNs in general can perform photorealistic style transfer (or color style transfer) faithfully with few artifacts.[1], [2] One group demonstrated that color style transfer can be used for many applications, such as low-light image enhancement, underwater image correction, image dehazing, and image harmonization, which are similar tasks to what we attempt to address in our pre-processing steps.[3] A recent study employed a heuristic algorithm to normalize a set of input dermatology or digital pathology images that serve as the target domain during the training process for their GAN model.[4] This GAN model was used to achieve consistent color and illumination normalization across highly variable frames

of tissue. Our process would be to utilize our “reference” color distribution as the target domain for a similar model, utilizing either a GAN or an architecture similar to the Neural Preset paper ([3]) to achieve real-time color normalization without computationally expensive pre-processing.”

- [1] H. Huang *et al.*, “Real-time neural style transfer for videos,” in *Proceedings - 30th IEEE Conference on Computer Vision and Pattern Recognition, CVPR 2017*, 2017, vol. 2017-January. doi: 10.1109/CVPR.2017.745.
- [2] J. Li, K. A. Skinner, R. M. Eustice, and M. Johnson-Roberson, “WaterGAN: Unsupervised generative network to enable real-time color correction of monocular underwater images,” *IEEE Robot. Autom. Lett.*, vol. 3, no. 1, 2018, doi: 10.1109/LRA.2017.2730363.
- [3] Z. Ke, Y. Liu, L. Zhu, N. Zhao, and R. W. H. Lau, “Neural Preset for Color Style Transfer,” in *Proceedings of the IEEE Computer Society Conference on Computer Vision and Pattern Recognition*, 2023, vol. 2023-June. doi: 10.1109/CVPR52729.2023.01362.
- [4] M. Salvi, F. Branciforti, F. Molinari, and K. M. Meiburger, “Generative models for color normalization in digital pathology and dermatology: Advancing the learning paradigm,” *Expert Syst. Appl.*, vol. 245, 2024, doi: 10.1016/j.eswa.2023.123105.

3) dsWLC overcomes theoretically one of the main problems of BLC. Namely, the chance to move to or from dsWLC to WLC anytime during the procedure. What about photo bleaching?

White light causes rapid elimination of PpIX; therefore, photobleaching is possible when WLC is used for prolonged duration in PDD-assisted TURBT procedures. Photobleaching during BLC can increase the chance of missed tumors. In a recent review paper, the fluorescence was reported absent due to photobleaching after 30 minutes of WLC [1].

We acknowledge this limitation of BLC and wish to clarify that in our study we aimed to minimize the impact of photobleaching on the accuracy of our ground truth dataset. Our clinicians performed a quick survey of the bladder with blue light and white light, collecting two-minute videos at the beginning of each procedure, therefore minimizing the likelihood of photobleaching in the collected BLC images.

We updated in our manuscript to clarify this procedure in Methods section (page 10, line 285):

“Under approval from the Vanderbilt University Medical Center Institutional Review Board, we recruited 31 patients who were scheduled for a transurethral resection of

bladder tumor (TURBT) procedure with CYSVIEW at Vanderbilt University Medical Center. From each patient, we collected a few short video clips (around one minute in length) **at the beginning of the procedure**. Each video clip contained both WLC and BLC images since the standard cystoscopy procedure requires switching between the two modalities for bladder visualization."

[1] Mulawkar PM, Sharma G, Tamhankar A, Shah U, Raheem R. Role of Macroscopic Image Enhancement in Diagnosis of Non-Muscle-Invasive Bladder Cancer: An Analytical Review. Front Surg. 2022 Feb 21;9:762027. doi: 10.3389/fsurg.2022.762027. PMID: 35265660; PMCID: PMC8898829.

Notably, photobleaching will not be an issue for the proposed dsWLC technology because it is associated with the use of a dye (PDD); in our proposed workflow, there would be no dye – the use of dye is replaced by our digital staining network. Thus, there would be no photobleaching in the actual clinical workflow of the proposed technology. This difference allows dsWLC to gain an additional advantage over BLC: since photobleaching leads to under-detection and lower sensitivity, dsWLC may actually be a more sensitive solution than BLC.

Reviewer #2 (Remarks to the Author):

The paper is interesting and well written. It is obviously a step forward in diagnosis, but of course not as efficient as it should be on long term. Nevertheless the paper has the merit to be a preliminary work. I think that accuracy is still not extremely satisfying.

We thank the reviewer for finding merit in our study. As we continue to investigate on this topic, we hope to bring in additional study designs and methods to further establish the efficiency and accuracy of the proposed method.

The shown pics are very convincing, but it would have been nice to show some pics which were more doubtful in order to evaluate the problems in daily practice.

There are two possible interpretations of the reviewer's comment regarding "more doubtful" pictures, so in the comment below, we try to address both:

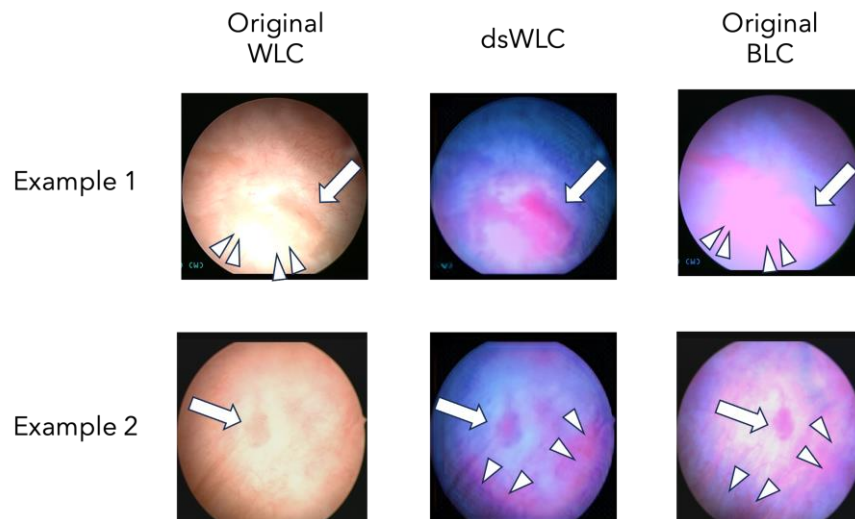
1. The eventual goal of our study is to bring additional accuracy in the detection of "more doubtful" tumors, such as CIS, in an office setting without the need for BLC equipment. However, in this proof-of-concept study, biopsy samples were not collected as a part of the study, and therefore, we cannot definitively declare that dsWLC has an advantage over WLC in such cases. We have included some

discussion on our future steps to collect clinical confirmation on page 9 lines 275-277 of the revised manuscript.

“We expect that future efforts to recruit more patients and obtain histological information can lead to increased accurate labeling of CIS tumors, contributing to solving one of the most vexing problems in bladder cancer detection. **In future studies, the collection of larger patient datasets and tissue type confirmation from biopsy will enable calculation of true sensitivity and specificity metrics for the dsWLC approach that can be compared against standard white light imaging.**”

In an effort to provide “more doubtful” pictures, in the revision, we include a new supplementary figure that provides examples of dsWLC showing fluorescence on flat, tumor-looking lesions that may be CIS. We have added a reference to this new figure in the updated main manuscript, in lines 164-165, page 6:

“Realistic bladder wall appearance was preserved and comparable fluorescent intensities to color-normalized ground truth BLC images were generated. Videos showing the original WLC, BLC and the dsWLC of an example bladder region are reported in the Supplementary Materials. **Additional examples of flat lesions are included in Supplementary Figure 1.**”



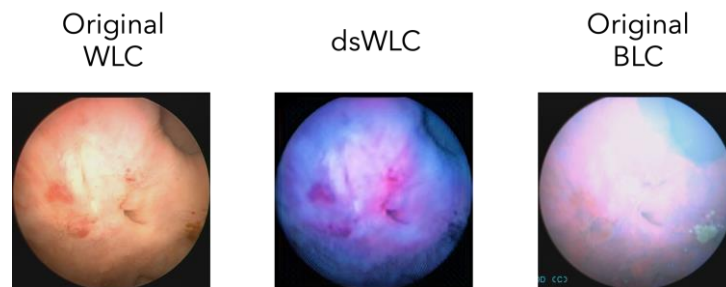
“Supplementary Figure 1. Two examples of unregistered WLC, dsWLC, BLC image pairs showing the staining performance of digital staining on possible flat tumors (without histology confirmation). Example 1 shows a flat lesion that is easy to miss under WLC due to image saturation and lack of contrast, but visible under dsWLC and BLC imaging. The original BLC image shows a large fluorescent area near the bottom left corner, which could be a saturation artifact. Example 2 shows a flat lesion surrounded by inflammation, lacking contrast in WLC but visible under dsWLC and BLC. White arrows = concern for CIS (flat

lesions). White triangles = likely benign findings, such as inflammation or saturation artifact. ”

2. If by “more doubtful” the reviewer is referring to inaccurate digital staining results and is suggesting that we should include examples of unsuccessful staining, we note that we already included such an example in Figure 7 in the original manuscript. The example highlights both areas of insufficient fluorescent staining compared to the BLC image as well as false fluorescence (fluorescence missing from the BLC image) possibly because of saturation. In addition, BLC is known to have false positives both due to certain camera angles and after extended instillation time. While we avoided including obvious cases of false fluorescence in ground truth BLC images, some features of false fluorescence did appear in our resulting dsWLC. We have added a new supplementary figure for this resubmission, showing two questionable cases.

In the Results section of the revised main manuscript, line 189 page 7:

“False fluorescence in the clinical, ground truth BLC is also known to be commonly found at the tangential bladder wall,³² and thus can lead to “false negative” regions when comparing the fluorescent masks of the dsWLC to the ground truth (row 4), although these are likely true negatives. Additional examples can be found in Supplementary Figure 2.”



“**Supplementary Figure 2.** Example of inaccurate digital staining results. Inflamed bladder tissue was highly fluorescent under BLC but lacking sufficient contrast and fluorescence in dsWLC. Example BLC shown here is original cystoscopy frames without green hue correction. The differences in colors that can be observed here are resulting from the green hue correction step in the preprocessing pipeline, where we color-correct BLC images with such artifacts to the reference color palette. ”

It would also be nice to say some words about the ground truth, which is always as good as the people you chose.

Here in this proof-of-concept study, the ground truth we used were BLC images collected from nearly matched regions to several of the collected WLC images. As acknowledged in our response to reviewers' previous comments, there are a number of limitations to only using BLC as the ground truth. "People" factors include variations in the individual uptake of the dye, the overall level of inflammation in the bladder, the clarity of the fluid that distends the bladder, etc. It is worth noting that we were intentional to include some representative cases like these in our training datasets to establish a robust network (both for color normalization and style transfer) that is suitable for handling real-life cystoscopies. To address this comment, we added the following to the Discussion section, in lines 238-241 page 8-9:

"In our study, we included cystoscopy images with varying illumination conditions and redness levels in the dataset to represent real world clinical cases and to establish our processing and network to robustly handle input cystoscopy video frames, aiming for successful clinical translation of the technology."

Given the pretty complicated procedure, I am not sure, whether this technique can be easily implemented into daily practice as it is at the moment.

We thank the reviewer for this comment. A similar question was also brought up by reviewer 1. We believe that real-time implementation of the proposed method is possible. Through a joint neural network that connects the color normalization step and the unpaired image style transfer, clinicians would be able to seamlessly switch between WLC and dsWLC in software during the procedure, as they do now for standard BLC. In fact, because the images are generated digitally, it will be possible to show both views simultaneously, which may be an advantage over current BLC workflows. We include the following paragraph in the revised manuscript, in lines 241-255 page 9, which describes the potential for real-time performance:

"In addition, real-time conversion of WLC into dsWLC is an important consideration to ensure practicality of this approach. To enable clinical translation of the proposed technology, a future task would be to address the most time-consuming step in the pipeline: color adaptation. Several groups have suggested that real-time color adaptation is possible. GANs, SSL (self-supervised learning) models, and CNNs in general can perform photorealistic style transfer (or color style transfer) faithfully with few artifacts.[1], [2] One group demonstrated that color style transfer can be used for many applications, such as low-light image enhancement, underwater image correction, image dehazing, and image harmonization, which are similar tasks to what we attempt to address in our pre-processing steps.[3] A recent study employed a heuristic algorithm to normalize a set of input dermatology or digital pathology images that serve as the target domain during the training process for their GAN model.[4] This GAN model was used to achieve consistent color and illumination normalization across highly variable frames of tissue. Our process would be to utilize our "reference" color distribution as the target domain for a similar model, utilizing either a GAN or an architecture similar to the

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Reviewer #1 (Remarks to the Author):

The paper has been improved significantly.

We thank the reviewer for acknowledging the improvement we made in the revised manuscript.

Reviewer #2 (Remarks to the Author):

The paper is interesting and well written and sticks to modern concepts.....It is a shame that the relation with histology was not done.

The authors could maybe insist a bit more on Cis and atypical changes in the bladder and their "trouble" with this

We thank the reviewer for seeing merits of our paper. In this design of the current study, histology confirmation was not necessary to establish the digital staining network, which serves to create BLC-mimicking images from WLC data. Given the promising results of the current study, we propose to include histological information in future studies, as we believe the inclusion of this information would provide the clinical validation of the proposed digital staining method and is likely to further improve the network.

On page 15 and 16, line 439 to 485, we added the following discussion:

“The current work aims to recreate blue light images, but it is well-known that blue light imaging suffers from a high level of false positives caused by inflammation and irritations in the bladder.³⁴ Here, we have recreated the results of current BLC images, which means our digitally stained outputs may similarly demonstrate a high false positive rate if tested on clinical samples. We hypothesize, however, that a more comprehensive deep learning digital staining model may outperform standard BLC imaging and effectively avoid rendering benign tissues as fluorescent, since cancerous lesions have distinct textures (carpet-like or papillary appearance) and colors from the normal and benign bladder wall.^{4,35} ... In future experiments, original image sizes (1024 by 1024) can be maintained to capture finer structural features to achieve better staining accuracy. Furthermore, we believe that the model would be highest-performing when training images would be combined with histology information. Such a model, however, would likely require biopsy sample collection and access to histological confirmation of the obtained tissue type. The current study, which aimed only to recreate BLC appearance, did not obtain histological confirmation;

instead, our assessment of the model output relied on BLC images as the assumed ground truth, which is a low-specificity imaging modality, as discussed above. Future studies involving the histology data could combine both the generative step converting WLC to BLC and a classification step to identify and highlight tumors, especially those CIS tumors which have been known as challenging to detect under WLC alone.

... The promising results shown in this study motivate a larger clinical study. We expect that future efforts to recruit more patients, providing an increased variety in tumor morphology, and to obtain histological information would lead to increased accurate labeling of CIS tumors, contributing to solving one of the most vexing problems in bladder cancer detection. In future studies, the collection of larger patient datasets and tissue type confirmation from biopsy will enable calculation of true sensitivity and specificity metrics for the dsWLC approach that can be compared against standard white light imaging.”