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# Unexpected endoscope surface phenomena in soaking mode of automated endoscope reprocessor: fluorescent tracer redeposition and persistent surface-tension bubbles

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**Abstract:** With the widespread use of endoscopy, there has been increasing concern about endoscope-associated pathogen transmission. In this context, the impact of different automated endoscope reprocessor (AER) operating modes on endoscope external surface cleaning remains underexplored. We compared three AER modes (soaking, jetting, and jetting-soaking) and observed two unexpected physical phenomena unique to the soaking mode. First, fluorescent tracer revealed thicker, linear deposition patterns on the external surface, whereas the jetting-containing modes produced a thinner and more even surface distribution. Second, the soaking mode generated numerous persistent surface-tension bubbles that remained attached to the external surface throughout the reprocessing cycle. Despite these differences, all three operating modes passed residual protein testing and yielded negative bacterial cultures, indicating that the soaking mode met current microbiological benchmarks under the tested conditions. To our knowledge, this study is the first to document such visible and reproducible physical phenomena during AER reprocessing. Although these findings do not indicate immediate reprocessing failure, they suggest that external surface fluid dynamics may be an underrecognized factor in AER mode optimization and warrant further investigation under more challenging real-world conditions.

**Key words:** Endoscope reprocessing; Automated endoscope reprocessor; Soaking mode; Surface disinfection; Fluorescent tracer; Surface-tension bubbles



With the widespread adoption of flexible endoscopy in diagnostic and therapeutic practice (ASGE Standards of Practice Committee et al., 2025; Barkun et al., 2025; Dinis-Ribeiro et al., 2025), endoscopy-associated pathogen transmission has been a growing concern (Houri et al., 2022; Ofstead et al., 2025). This issue is of particular interest in therapeutic procedures such as endoscopic retrograde cholangiopancreatography, where disruption of the mucosal barrier may expose injured tissue (Cissé et al., 2026). In patients with pre-existing mucosal injury, even diagnostic endoscopy may carry a transmission risk if residual contamination on the endoscope surface contacts compromised mucosa (Day et al., 2021).

On the other hand, attention in endoscope reprocessing has traditionally focused on internal channels (Du et al., 2024; Liu et al., 2026). However, the external surface of the endoscope is also clinically important: during procedures, the insertion tube can be contaminated by blood, body fluids and intestinal flora from the patient

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(Rutala and Weber, 2023). It may also acquire contamination through contact with gloves, bedside equipment, and the surrounding environment. Studies have underscored that used endoscopes can carry a substantial pathogen burden and that reprocessing-related handling may contribute to environmental contamination and droplet dispersal, highlighting the potential role of external surfaces in cross-transmission (Ayres et al., 2023; Rao et al., 2025).

Automated endoscope reprocessors (AERs) are now widely used in endoscope reprocessing and have become routine for cleaning and high-level disinfection (Day et al., 2021). Common AER operating modes include soaking, jetting and jetting-soaking. Because internal channel disinfection relies mainly on the circulation of disinfectant through the lumen (Liu et al., 2026), exposure within the channel is relatively standardized. In contrast, the outer surface depends on external contact with the disinfectant and may therefore be more sensitive to differences among operating modes. Furthermore, the irregular contours, grooves and protruding structures of flexible endoscopes may affect surface coverage and contact time, raising questions about whether these modes provide equivalent surface coverage and cleaning.

During the comparison of three modes under identical total cycle times, we observed two unexpected physical phenomena that occurred in the soaking mode. These findings came from separate experimental conditions and are reported here for their potential practical relevance.

This experimental study comprised three parallel experimental arms evaluating the reprocessing effectiveness of three AER operating modes (jetting, soaking, and jetting-soaking) on the external surface of endoscopes. The outcomes included reprocessing solution distribution, cleaning efficacy, and disinfection efficacy. Each mode was tested in six independent runs (three replicate runs per gastroscope × two gastroscopes in rotation). A schematic overview of the experimental design is provided in Fig. S1.

Three AERs (Hongkang Kairui, China) and the same peracetic acid disinfectant (Hongkang Kairui, China) were used in the experiments. All AERs were confirmed to be functioning properly before testing. In the jetting mode, a rotating arm above the endoscope continuously sprayed disinfectant onto the external surface; in the soaking mode, the chamber was filled from the bottom until the endoscope was fully immersed, without jetting; in the jetting-soaking mode, disinfectant was first introduced through the jetting nozzle. Once the preset liquid level was reached, the endoscope remained immersed while the jetting arm continued to rotate.

For the reprocessing solution distribution assessment, a fluorescent suspension was prepared by dissolving a fluorescent tracer at 60 g per 15 L water in place of the conventional reprocessing solution. Only the disinfection step was performed for this test. After cycle completion, two investigators independently assessed the distribution and uniformity of fluorescent coverage on the external surface under ultraviolet light. To determine whether the entire surface had been uniformly wetted, surface wetness was also visually assessed under standard room lighting. Any disagreements were resolved by consensus.

For the cleaning assessment, 3 mL of sterile calf serum was evenly applied to the entire external surface of the insertion tube and allowed to air-dry at room temperature for 10 minutes. After the completion of the cleaning step, residual protein was sampled from the entire external surface using a Clean-Trace Surface Protein swab (3M, USA). Protein residue was assessed semiquantitatively using the manufacturer's color chart: green was considered pass, whereas gray, light purple and dark purple indicated increasing residual protein.

For the disinfection assessment, 3 mL of the *Pseudomonas aeruginosa* suspension ($10^6$ CFU/mL) was evenly applied to the entire external surface of the insertion tube using sterile cotton swabs. The inoculated endoscope was then air-dried at room temperature for 10 minutes. After completion of the full reprocessing cycle, the external surface of the insertion tube was sampled with sterile pre-moistened swabs and submitted to the microbiology laboratory for culture and analysis.

Fluorescent tracer assessment showed complete coverage of the outer surface of the endoscope in all three operating modes (Fig. 1). However, the pattern of fluorescent distribution differed across groups. In the soaking mode, thick, linear fluorescent deposits could be observed on the external surface. These deposits appeared as streaky patches, suggesting that as the endoscope sat in static fluid, tracer aggregates resettled onto the surface. In contrast, the jetting mode produced a thin, uniform fluorescent layer with no visible aggregates, indicating

that mechanical flushing effectively removed or redistributed the material. The combined jetting-soaking mode showed an intermediate pattern, with less aggregation than soaking alone but not as uniform as the pure jetting mode (Figs. 1a-c).

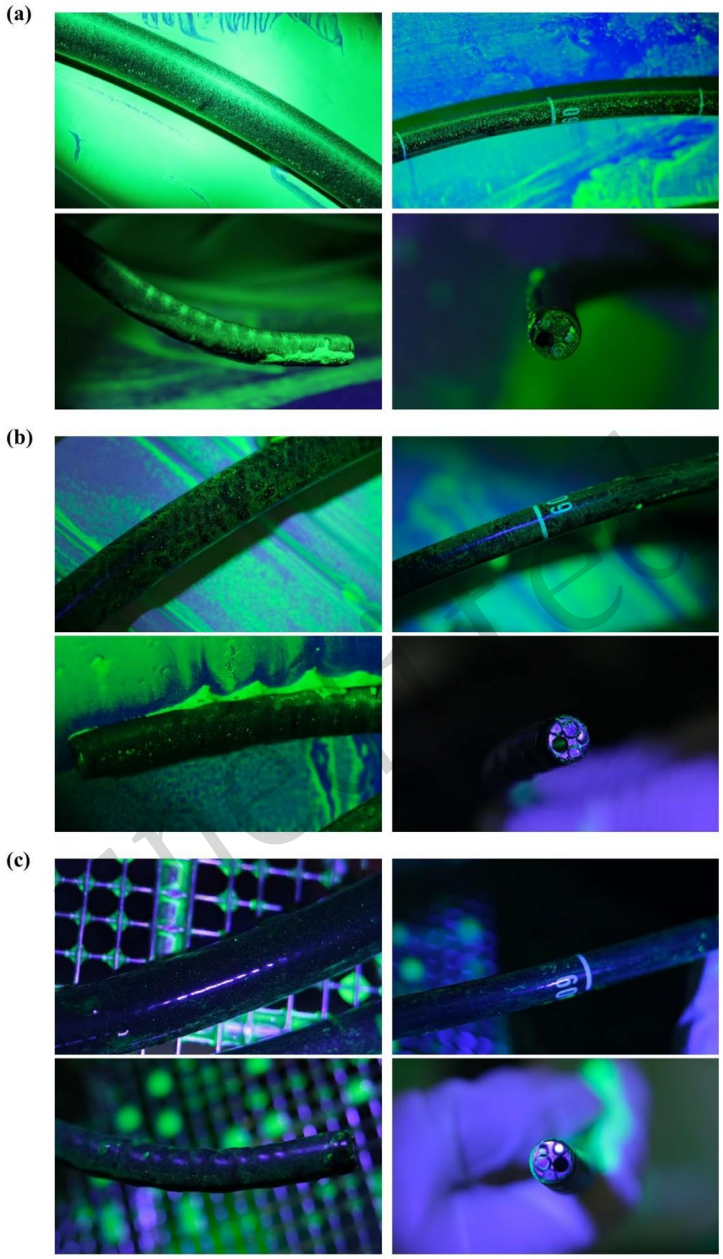


**Figure 1. Fluorescent tracer distribution on the endoscope external surface for the three AER operating modes. (a) Soaking mode: thick linear fluorescent deposits; (b) Jetting-soaking mode: intermediate pattern; (c) Jetting mode: thin, uniform layer. In each of panels (a), (b), and (c), the four sub-images display different parts of the endoscope: the top-left shows the universal cord; the top-right shows the middle section of the insertion tube; the bottom-left shows the distal tip of the insertion tube; and the bottom-right shows an end-on view of the distal tip revealing the lumen openings.**

This finding is noteworthy because it highlights a phenomenon by visual means that has rarely been discussed in the endoscope reprocessing literature. Under the soaking-mode condition, despite circulating flow within the internal channels, fluorescent tracer on the external surface exhibited a redistribution pattern con-

sistent with possible redeposition. Meanwhile, because the fluorescent powder served only as a surrogate marker, these observations cannot be taken as direct evidence of microbial or proteinaceous residue transfer; nevertheless, they raise the possibility that soaking mode may permit redeposition under certain conditions.

During these experiments, numerous persistent bubbles adhering to the external surface were incidentally observed throughout the cycle in the soaking mode (Fig. 2). The bubbles were present over the entire surface rather than confined to specific grooves or irregularities, and they varied from pinhead-sized to several millimeters in diameter. The bubble phenomenon was consistently reproduced across all six independent runs of the soaking mode.

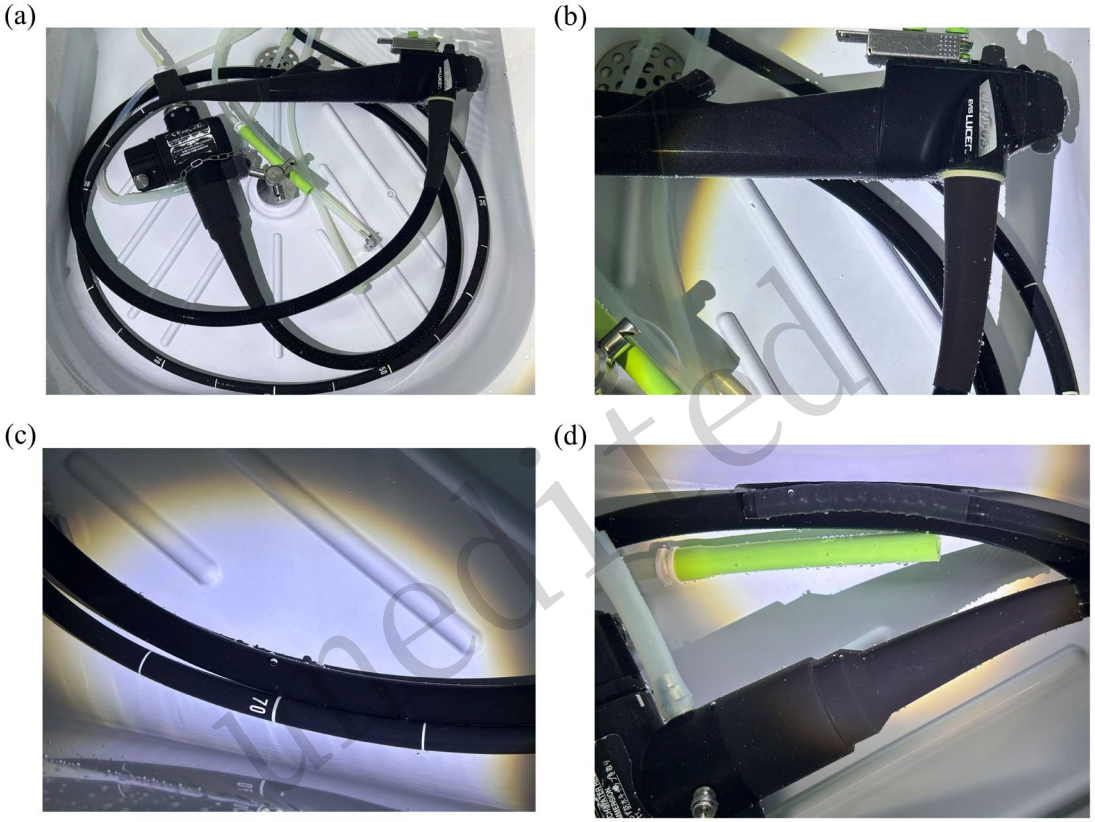


**Figure 2. Surface tension bubbles observed on the external surface of the endoscope in the AER soaking mode. (a) Overall view of the endoscope placed in the AER chamber; (b) Close-up of the control handle section; (c) Close-up of the middle section of the insertion tube; (d) Close-up of the distal end and the junction between the light guide connector and the universal cord. Bubbles varied in size and adhered to the entire endoscope external surface.**

Importantly, despite the presence of these bubbles and the visible fluorescent aggregates in the soaking mode, all samples in all three modes passed the residual protein test and showed no bacterial growth on culture (0 CFU). Thus, neither the bubble retention nor the fluorescent aggregates led to any detectable reprocessing failure under our experimental conditions.

Under the standardized low-to-moderate bioburden challenge, all modes achieved complete protein clearance and high-level disinfection against *P. aeruginosa*. This demonstrates that, from a strictly microbiological standpoint, the static soaking mode remains a technically valid and clinically acceptable option under the conditions tested, and our observations should not be interpreted as an immediate safety warning for the current clinical practice.

Nevertheless, the striking physical differences in fluorescent tracer distribution warrant attention from a fluid-dynamic and quality-assurance perspective. Because the fluorescent powder served as a non-adherent particulate surrogate, the streak-like aggregates observed in the soaking mode indicate gravitational settling and

possible redeposition of the detached particles onto the external surface in the absence of external fluid agitation. By contrast, the jetting mode continuously applied directional shear stress via the rotating spray, which not only delivered the disinfectant but also facilitated the downward transport and drainage of dislodged particulate matter, resulting in a thin, homogeneous film. This issue could become more relevant under conditions not examined in the present study, such as a higher initial bioburden, suboptimal pre-cleaning, or aged or damaged endoscope surfaces.

Similarly, although no microbiological failure was detected in our experiments, the presence of persistent bubbles suggests the existence of a physical phenomenon. These bubbles either did not cover a sufficient surface area to compromise biocidal action, or a thin liquid film phase beneath the bubbles still allowed adequate disinfectant contact. Nonetheless, their presence underscores the lack of mechanical disturbance in static immersion and serves as a visual indicator of an existing physical limitation. Persistent surface-tension bubbles could theoretically reduce direct disinfectant contact with bubble-covered areas if present to a sufficient extent. This may warrant further investigation, particularly in real-world settings where endoscopes undergo repeated use and surface integrity may change over time.

From a practical perspective, our findings support a stratified recommendation. Although the jetting and combined jetting-soaking modes are increasingly adopted in some settings, static soaking remains widely used in real-world AER practice. Because this mode does not require continuous spray circulation, it may need fewer consumables and is associated with lower operating costs, which may partly explain its continued widespread use. Accordingly, the redeposition and persistent bubble phenomena observed under the soaking mode remain clinically relevant, particularly in centers where this mode is routinely employed.

Although all modes appear to satisfy baseline safety requirements, the jetting mode provides greater physical uniformity and mitigates particulate redeposition. We therefore propose that, where clinically appropriate and logistically feasible, AER settings incorporating active external jetting should be preferentially adopted, particularly for endoscopes used after heavy contamination or therapeutic procedures. For institutions in which soaking is the only available option, or in those where it is selected for resource conservation, our findings do not necessarily warrant the immediate replacement of existing systems; rather, they provide a mechanistic basis for a gradual transition to dynamic modes as resources permit, while also underscoring the importance of rigorous pre-cleaning when the soaking mode is used.

To our knowledge, this is the first report to describe fluorescent tracer redistribution and persistent surface-tension bubbles as physical phenomena observed under soaking-mode conditions in an AER. Although all three AER modes met the microbiological and residual protein endpoints evaluated in this study, these phenomena were not observed under jetting mode conditions in our experiments. These findings may be relevant when selecting AER operating modes, particularly in settings involving higher bioburden or compromised device surfaces; however, their practical significance remains to be established through further microbiological studies.

Only gastroscopes were evaluated in the present study. This is because as one of the most commonly used endoscope types, gastroscopes provide a practical model for the initial assessment of AER performance. In addition, their relatively simple external geometry may have reduced device-related structural variability, thereby allowing clearer observations of tracer deposition and bubble distribution patterns. Nevertheless, the generalizability of these findings to more complex devices, such as duodenoscopes or echoendoscopes, remains uncertain. In fact, these phenomena could be more pronounced in such endoscopes because devices with more intricate external geometries may pose greater hydrodynamic challenges during AER. However, this possibility requires direct investigation.

In conclusion, we identified a reproducible pattern of fluorescent tracer redistribution and persistent surface-tension bubbles under soaking-mode AER. Although these observations were not associated with the non-attainment of microbiological or residual protein endpoints in the present model, they raise important questions regarding the potential physical effects of soaking mode on the endoscope exterior and therefore warrant further investigation. These effects should be tested by incorporating more challenging contamination

models, aged or damaged endoscope surfaces, additional controls, and direct chamber sampling.

## Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## Author contributions

Mei LI performed conceptualization, investigation, methodology, formal analysis, writing of the original draft, and visualization. Chen-Ying ZHOU performed investigation, data curation, validation, and writing - review and editing. Ting TENG performed data curation. Qing GU performed conceptualization, project administration, and supervision. All authors have read and approved the final manuscript, and therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

## Compliance with ethics guidelines

This study did not involve human subjects, animal experiments, or any clinical samples requiring ethical approval. No patient data or identifiable human materials were used. Therefore, institutional review board approval was not required.

## Declaration on the use of generative AI tools

During the preparation of this work, the authors used DeepSeek for language polishing. The input prompts used included: "As a native English-speaking editor for a medical SCI journal, please help me check whether this passage contains any grammatical or expression errors.". The use of this tool/service occurred on 2026/06-2026/07. Relevant records, generated content, and the modification process have been preserved and are available for editorial review if requested. The output generated by this tool was thoroughly reviewed and edited by the authors to ensure accuracy, originality, and compliance with journal standards. The authors take full responsibility for the final content of this manuscript as submitted.



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**Supplementary information**

Fig. S1

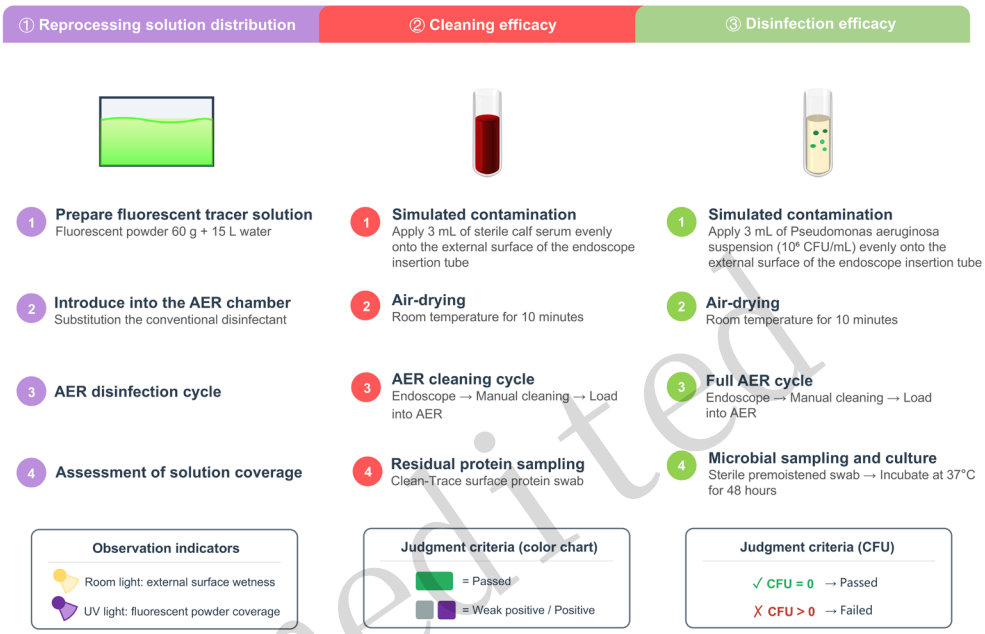


Figure S1. Schematic overview of the experimental design