



# CosMx<sup>®</sup> Best Practices

Volume IV

# Tips to optimize sample prep for highly autofluorescent samples on CosMx<sup>®</sup> SMI

The CosMx<sup>®</sup> Spatial Molecular Imager (SMI) instrument performs fluorescent imaging to read out in-situ hybridized fluorescent reporters which uniquely identify RNA transcripts. The presence of tissue autofluorescence background may reduce detection efficiency of transcripts. CosMx automatically performs pre-bleaching of the slide to mitigate tissue autofluorescence, by controlling an on-instrument light source based on a user-selectable “Pre-bleaching Profile”. The pre-bleaching selection corresponds to different durations of photobleaching exposure (default is ‘Configuration C’, 60s per FOV). See table 6: Pre-bleaching Profile in the CosMx SMI Instrument User Manual (MAN-10161-09, <https://university.nanosttring.com/cosmx-smi-instrument-user-manual/1447795>)

In this post, we share two additional methods to optimize CosMx sample prep on tissues known to be highly autofluorescent in cases where even the highest pre-bleaching profile on the instrument (‘Configuration B’, 90 seconds) is deemed insufficient. In these rare cases, the following additional measures may be experimented with, independently or in combination, to reduce autofluorescence on the tissue.

## Tip 1: Chemical bleaching during sample preparation

The Appendix of the CosMx SMI Manual Slide Preparation for RNA Assays (MAN-10184-05, <https://university.nanosttring.com/cosmx-smi-manual-slide-prep-for-rna-assays/1831024>) includes information for working with challenging tissues. In the section “High Autofluorescence in Tissue (RNA protocol)” (page 114), a protocol is described using 0.05% H<sub>2</sub>O<sub>2</sub> to reduce autofluorescence prior to the Target Retrieval step on Day 1.

One way to further optimize this sample preparation step is to experiment with increasing the H<sub>2</sub>O<sub>2</sub> concentration beyond the value indicated in the manual appendix. A range of 0.05 – 0.25% was found to be effective at reducing autofluorescence with minimal negative effects on tissues. In general, chemical bleaching has been shown to reduce false codes by about 50% in high autofluorescence tissues; however, this method may also reduce RNA counts.

This method should be used in conjunction with photobleaching (on the instrument or Benchtop).

## Tip 2: Benchtop Photobleaching during slide preparation

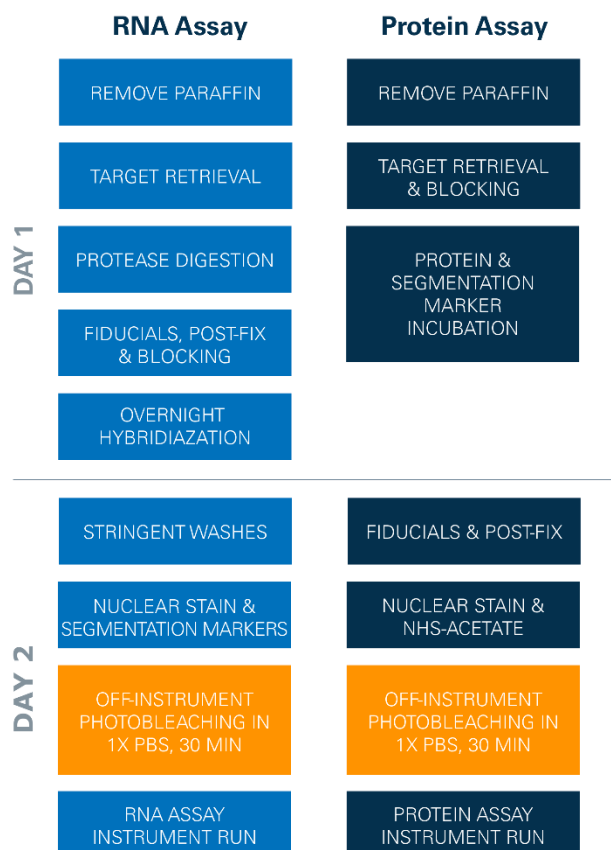
A benchtop photobleaching instrument may provide an alternative method to control the exposure duration of light on particularly challenging tissues. As the dedicated device exposes the full slide simultaneously with broad band illumination, rather than on a per FOV basis as performed by the CosMx SMI, higher doses of photobleaching across more visible wavelengths can be delivered in much shorter time. This method may also be used as a replacement for the on-instrument pre-bleaching profile to increase effective instrument throughput (by 5-10% on RNA and up to 33% on Proteins).

## Required equipment and materials:

Equipment and Materials	Source	Part Number
1X PBS - diluted 1:10 from Phosphate-Buffered Saline (10X) pH 7.4, RNase-free	ThermoFisher Scientific® (or comparable)	Example: <a href="#">AM9625</a>
TiYO Autofluorescence Quenching System	Bulldog Bio, Inc. (or comparable)	Example: <a href="#">TiYO</a>

Perform after the “Nuclear and Cell Segmentation Staining” step on Day 2 (see page 95 of MAN-10184-05), Figure 2.

1. Add approximately 30-50mL of PBS to the sample setting area of the tray
2. Insert slide(s) into tray containing PBS, with the **tissue facing down**
3. Quench for 20 – 120 minutes, following instructions provided by the TiYO equipment
4. Remove slide(s) from TiYO and Resume the Slide preparation instructions (*see page 96 of MAN-10184-05*).
5. When performing the CosMx run, **select “None” for the pre-bleaching profile** if no additional on-instrument bleaching is required (*see page 36 of CosMx SMI Instrument User Manual* (MAN-10161-09, <https://university.nanostring.com/cosmx-smi-instrument-user-manual/1447795>). Selecting “None” for pre-bleaching profile will reduce the instrument run time by approximately 30-90 seconds per FOV.



**Figure 1.** Location of off-instrument photobleaching protocol in CosMx sample preparation.