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Headline: Optimising RNAscope® for Organotypic Cell Cultures

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RNAscope®, developed by Advanced Cell Diagnostics, Inc. (Newark, CA, USA), is a novel and increasingly popular technology for the *in situ* analysis of RNA within fixed tissues.[1] Without disturbing tissue morphology, it enables the localization of specific targets within RNA, examination at the level of the single molecule, and the simultaneous suppression of background noise. By applying RNAscope® to organotypic cell cultures (which use a three-dimensional organization of cells that more accurately represents the morphological, physiological, and molecular aspects of tissues), it has the potential to provide a window into gene expression as it occurs in the human body. This could have important implications in a number of fields, including molecular pathology, toxicological analysis, drug discovery, and drug development. In addition, since the approach relies exclusively on *in vitro* techniques and tissues provided by human donors, it supports the internationally recognized "3Rs" of animal research: "replacement, reduction, and refinement".[2]

Quantifying Optimization

We presented the results of a study which sought to determine the optimized conditions for RNAscope® and assess its ability to detect and visualize cell-specific RNA in human organotypic nasal epithelial cultures.[3] The experimental process was divided into three steps:

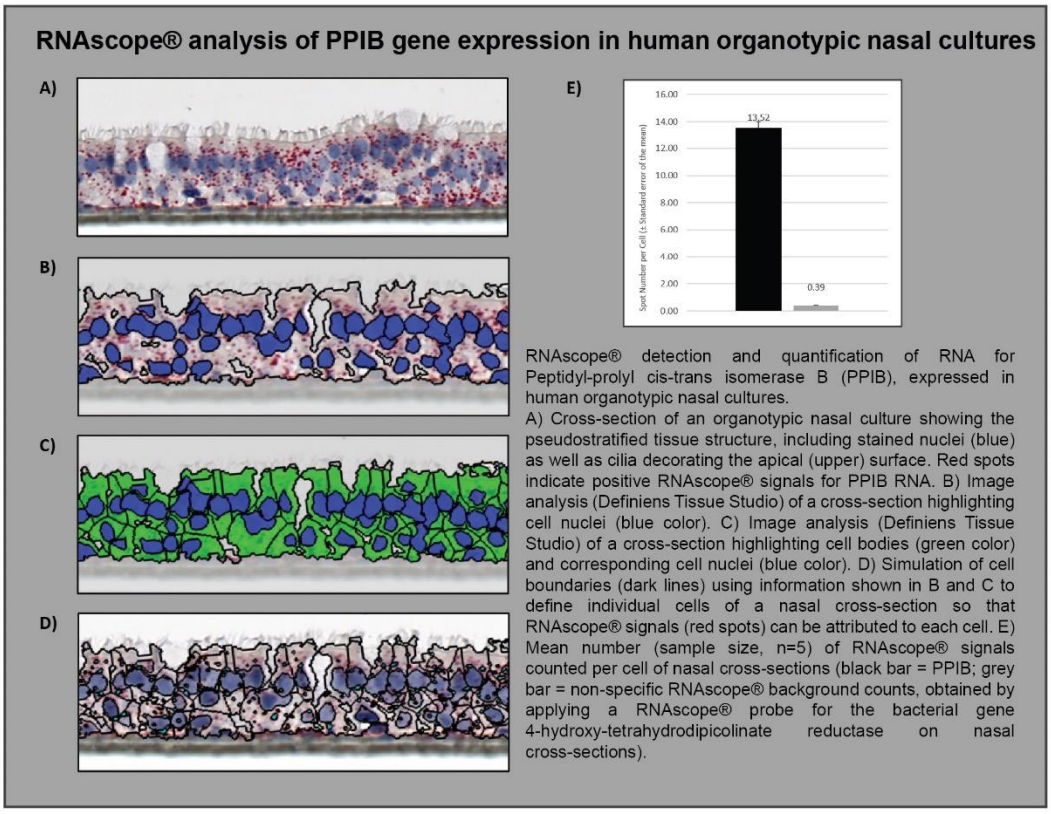
1. **Histology:** Slides containing five replicates of nasal cultures were stained to assess tissue morphology.
2. **Optimization:** Optimization focused on, i) pretreatment of cultures, ii) incubation with protease to unmask target RNA, and iii) amplification to increase sensitivity.
3. **Quantification:** Slides were scanned to create digital images. Quantification was then carried out with a custom-built solution designed by Definiens, AG. (Munich, Germany), involving the automated detection of relevant tissue areas, nucleus detection, cell simulation, cell and nuclei visualization, and spot detection.

A scoring system from zero to four, based on the number of spots per cell, was used to evaluate the results of the initial staining. Scores for positive and negative controls determined whether the RNAscope® technique was optimized (a probe detecting RNA of the human Peptidyl-Prolyl Cis-Trans Isomerase B (PPIB) gene was used as a positive control, while a probe for RNA of the bacterial gene 4-hydroxy-tetrahydrodipicolinate reductase (dapB) was used as a negative control). From the initial microscopic evaluation, a strong expression level of the positive control was observed with an absence of background noise, while no expression of the negative control was observed. The average quantification score for the positive control was

three, and for the negative control zero, confirming that the RNAscope® technology for human organotypic nasal epithelial cultures was optimized (low values of standard deviation suggested that there was no variability between the five replicates).

Applications

The study shows that RNAscope® can be optimized for the detection and visualization of cell-specific RNA in human organotypic nasal epithelial cultures, with no disturbance to the RNA or to tissue morphology. This is crucial, as preserving RNA and tissue morphology *in vitro* ensures an accurate representation of human biology. The technique has the potential to facilitate rapid, accurate, and reliable RNA detection and localization, and thus has important implications across a variety of fields in molecular biology. At Philip Morris International, for example, we have conducted a range of *in vitro* organotypic studies looking at the effects of the aerosols of Reduced-Risk Products* on epithelial cells of the bronchus, oral cavity, and nasal passages. RNAscope® may prove to be a valuable complement to the gene expression data generated from these studies.



About Laurent Neau

Laurent Neau is Lead Technician, Tissue Research Laboratory, Philip Morris International. He holds M. Sc. in Biotechnologies from Lille University, France. He has worked as a technician in histology labs for seven years in both academic research and pharmaceutical company settings. He has developed strong expertise in molecular biology, immunohistochemistry and *in situ* hybridization.

Where can readers find more information?

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* Reduced-Risk Products ("RRPs") is the term we use to refer to products that present, are likely to present, or have the potential to present less risk of harm to smokers who switch to

these products versus continued smoking. We have a range of RRPs in various stages of development, scientific assessment and commercialization. Because our RRPs do not burn tobacco, they produce far lower quantities of harmful and potentially harmful compounds than found in cigarette smoke.

References

1. Wang, F. *et al.* 2012. RNAscope: a novel in situ RNA analysis platform for formalin-fixed, paraffin-embedded tissues. *Journal of Molecular Diagnostics* 14(1), 22-9. Available online at: https://jmd.amjpathol.org/article/S1525-1578(11)00257-1/fulltext.
2. National Center for the Replacement, Reduction, and Refinement of Animals in Research. The 3Rs. Available online at: https://www.nc3rs.org.uk/the-3rs.
3. Neau, L. *et al*. 2018. Optimization and image analysis of RNAscope® technology on 3D human organotypic ciliated respiratory epithelial culture. 5th Digital Pathology Congress, Europe. 6-7 December 2018. London, UK.