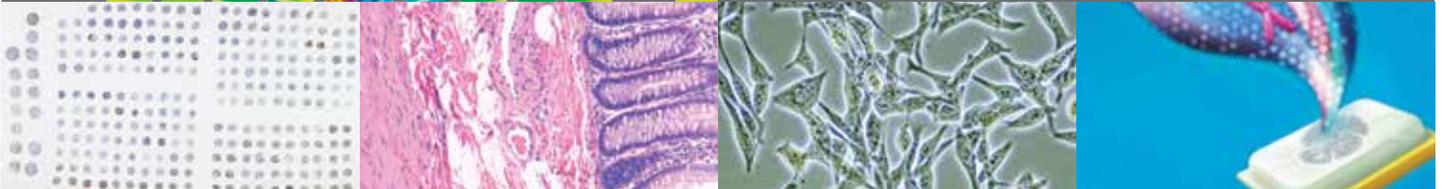
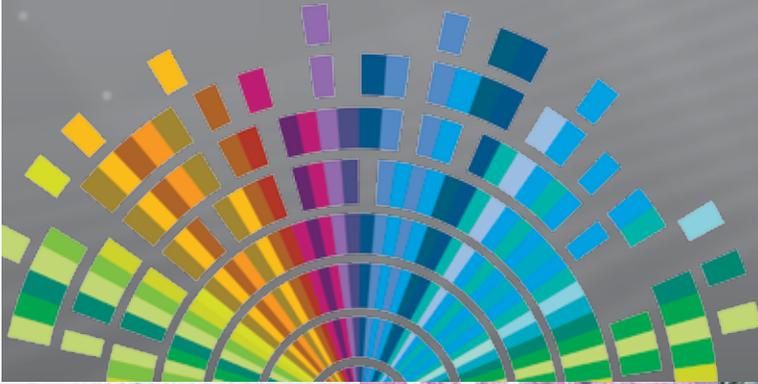




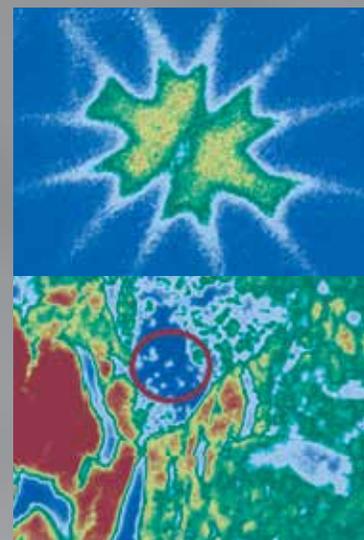
Agilent Sample Quality Control Solutions  
for Cell and Tissue Samples

# BETTER SAMPLES FOR BETTER RESULTS



## QUALITY STARTING MATERIALS FOR QUALITY RESULTS

Many biomarker discoveries fail at the validation stage. Although there may be several false positives from high throughput omics screenings, it is likely that many of these failures are a result of changes in sample quality and homogeneity. The National Institutes of Health (NIH) has recognized the importance of assessing sample preparation effects on biomarker studies and recommends that quality control (QC) metrics are incorporated early in the experimental process. A method that allows for prequalification of the samples used in discovery and validation studies is needed to address this discrepancy. Therefore, biomedical researchers recognize the need for quality control on samples upstream of omics technologies.



### **An ideal method to evaluate cell and tissue samples should:**

- Fit into existing workflows to minimize additional processing steps.
- Provide an objective and quantitative metric on the integrity of component being evaluated.
- Allow for fast and easy interpretation of results.
- Suit the objective of the assessment for resolving qualitative, quantitative or spacial issues.

Many of the tissue samples that are available to researchers in biobanks are formalin fixed paraffin embedded (FFPE) samples. Early characterization of FFPE tissue samples used for biomarker discovery and validation can reduce assay development time and decrease assay failure during late-stage testing.

This brochure details how you can use Agilent solutions to identify high-quality samples for your downstream analyses. Agilent makes solutions that enable researchers to address the challenges of prequalifying fresh, frozen, or FFPE samples upstream of omics-based workflows. You can find information on additional related products in our solutions web pages including PCR based mycoplasma testing, IHC reagents and molecular spectroscopy instruments.

## Agilent Microfluidics Products for Biomolecules

The Agilent Automated Electrophoresis Solutions offer fast and reliable separation of biomolecules with precise sizing and quantitation measurements. Obtain objective sample quality evaluation of total RNA and/or Genomic DNA integrity with these systems.

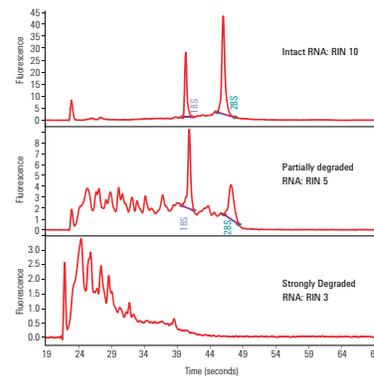
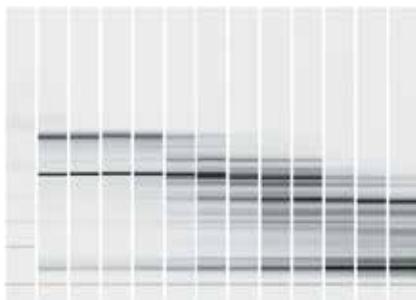
### Agilent 2100 Bioanalyzer system

With a track record of over 33,000 citations in peer-reviewed articles, the 2100 Bioanalyzer system is well established for measurements of DNA, RNA, and proteins in biological samples. The widely used RNA Integrity Number (RIN) provides an objective numeric quality assessment of total RNA samples.

Learn more at: [agilent.com/genomics/bioanalyzer](http://agilent.com/genomics/bioanalyzer)



Agilent 2100 Bioanalyzer system



A total RNA sample was degraded for varying times and the resulting samples were analyzed on the Agilent 2100 Bioanalyzer system using the Eukaryote Total RNA Nano assay. A shift towards shorter fragment sizes can be observed with progressing degradation.

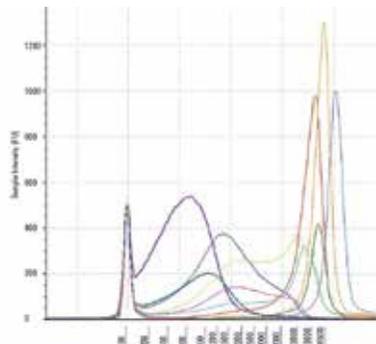
### Agilent 4200 TapeStation system

Use the 4200 TapeStation system to simplify your workflow with fully automated sample processing from 1 to 96 samples for DNA and RNA analysis. In addition to the RINe (RIN equivalent) for total RNA quality determination, this system also offers the Genomic DNA ScreenTape assay to enable reproducible and digital assessment of genomic DNA integrity with DIN (DNA Integrity Number).

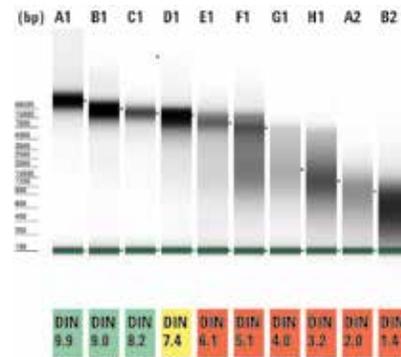
Learn more at: [agilent.com/genomics/tapestation](http://agilent.com/genomics/tapestation)



Agilent 4200 TapeStation system



Electropherogram Overlay of a gDNA degradation series.



Gel image gDNA samples showing a different degree of degradation and DIN values.

## NGS FFPE QC Kit with the AriaMx Real Time PCR System

### Accurately qualify and quantify amplifiable DNA in FFPE samples

Formalin-fixed paraffin-embedded (FFPE) tissue represents a valuable sample source for molecular cancer research. These samples provide a contextual snapshot of the tissue at a specific time point and stage of disease but are challenging to analyze.

The Agilent NGS FFPE QC kit is a qPCR-based assay that enables functional DNA quality assessment of input DNA prior to preparation of next generation sequencing libraries. This kit enables assessment of the integrity of DNA as well as accurate quantitation of amplifiable template going into library preparation. Furthermore, optimizations to the Agilent SureSelect<sup>XT</sup> and HaloPlex<sup>HS</sup> target enrichment NGS workflows based on the sample quality score are possible.

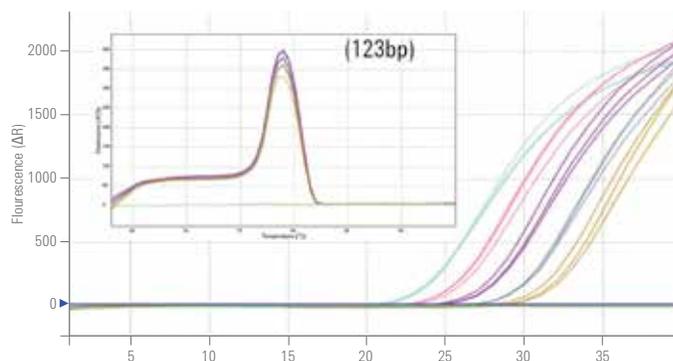
Together with the AriaMx Real Time PCR system, the NGS FFPE QC kit enables an end-to-end optimized solution for FFPE samples, from assessment of quality to library prep optimization, for increased probability of NGS library prep success, even for challenging archived samples.

Learn more at:

[agilent.com/genomics](http://agilent.com/genomics)



AriaMx Real Time PCR System



Standard curves and dissociation curves for the 123bp qPCR target used in the FFPE QC kit generated on the AriaMx Real Time PCR system.

## FTIR IMAGING - LABEL-FREE, NONDESTRUCTIVE CHEMICAL MICROSCOPY

### Image samples with automated tissue classification in your existing workflow

Fourier Transform InfraRed (FTIR) chemical imaging has been an important tool in analytical chemistry for almost 15 years, and has also been increasingly recognized as an important tool for life science research. FTIR imaging uniquely provides a rapid 2D total macromolecular snapshot of the sample, spatially resolved to a few microns across a large field of view.

FTIR imaging provides better tissue qualification with spatial resolution. FTIR imaging has been applied with great success in various academic research projects to provide information on tissue and cell type heterogeneity, and has been referred to as Spectral HistoPathology (SHP).

The challenges in tissue quality control require a solution providing spatially resolved information such as FTIR imaging in addition to other nucleic acid based QC metrics such as RIN and DIN. This optimized QC is able to identify specific tissue regions to avoid, such as in the case of necrotic regions. Alternatively, one can target a particular region of interest, such as epithelium.



FFPE Tissue Workflows: Traditional imaging (with stains) vs FTIR imaging (stainless)

### FTIR Imaging provides the following unique attributes for tissue sample QC

- Spatial resolution that accounts for tissue heterogeneity at the cellular level.
- Stainless/label-free and nondestructive so samples can be re-used for downstream analysis.
- Quantitative assessment of tissue/cell type that removes subjectivity.
- Automated analysis for high throughput screening.

Extensive literature exists demonstrating the unique capability of FTIR imaging for an objective and fully automated tissue classification. Therefore providing a rapid tissue prequalification for downstream omics analyses, ensuring that time and resources are devoted to tissue samples that contain the correct tissue types.



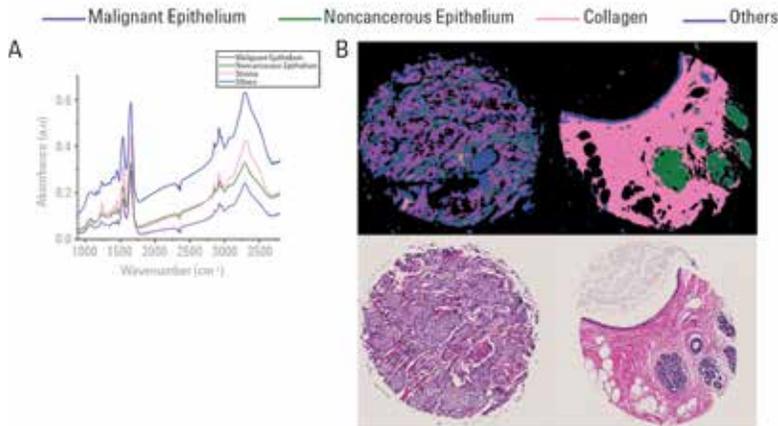
**Prof. Rohit Bhargava**  
University of Illinois

## FTIR imaging used to differentiate between malignant and non-malignant epithelium

Prof Rohit Bhargava, is a Bliss Faculty Scholar and Professor of Engineering at the University of Illinois at Urbana-Champaign. Prof Bhargava has pioneered the development of infrared spectroscopic imaging, starting from his doctoral thesis that was the first in this field .

*“FTIR imaging is the next generation imaging tool for studying both conventional histology and chemical composition of tissues. High-definition IR imaging now makes the quality of IR images close to that obtained from high end optical microscopes. While techniques like stainless staining can greatly reduce costs, increase multiplexed molecular analyses, and conserve tissue for analysis. FTIR chemical imaging before analysis can allow for quality control and assessment of pre-analytical variability”*

**PROF. ROHIT BHARGAVA, UNIVERSITY OF ILLINOIS**



Classes	Noncancerous epithelium	Malignant epithelium	Stroma	Others
Noncancerous epithelium	<b>95.64</b>	1.85	1.71	0.79
Malignant epithelium	5.08	<b>94.17</b>	0.16	0.59
Stroma	4.86	0.16	<b>94.47</b>	0.51
Others	2.75	0.45	0.71	<b>96.09</b>

FTIR imaging of breast tumors to distinguish malignant vs. non-malignant epithelium.

A: Average spectra of the regions of interest, manually marked on the samples for four different classes showing subtle but reproducible differences, reflecting the different chemical composition of the different classes.

B (top): Classified images of two samples using the random forest classifier with 10 decision trees.

B (bottom): The corresponding H&E stained images.

C: Confusion matrix computed by training a random forest classifier with 134 spectral features and 10 decision trees.

Reprinted and adapted with permission from Shachi Mittal, Tomasz P. Wrobel, L. Suzanne Leslie, Andre Kadjacsy-Balla, and Rohit Bhargava.

A four class model for digital breast histopathology using High-Definition Fourier transform infrared (FT-IR) spectroscopic imaging, Medical Imaging 2016: Digital Pathology, Proc. of SPIE Vol. 9791, 979118. Copyright (2016) SPIE

## FTIR imaging used to identify necrosis in tissues

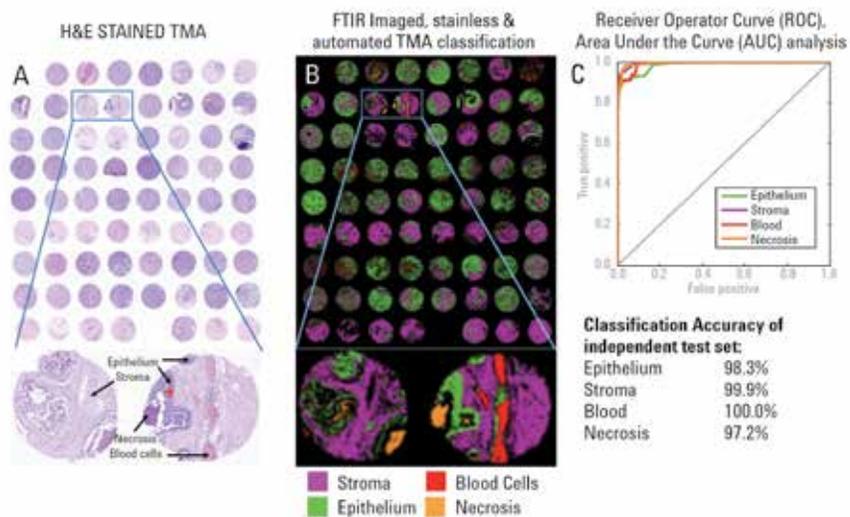
Peter Gardner is a Professor of Analytical and Biomedical Spectroscopy at the University of Manchester. His work focuses on the use of vibrational spectroscopy of complex samples with a focus on biomedical problems. In close collaboration with the Institute of Cancer Studies and the Christie Hospital in Manchester, he has worked on disease diagnostics, theranostics, and fundamental understanding of disease. He is a director of the International Society for Clinical Spectroscopy (CLIRSPEC).



**Prof. Peter Gardner**  
University of Manchester

*“FTIR imaging is a rapid, stain free, method of obtaining a chemical map of your sample. In the case of tissue studies, application of the correct chemometric analysis means that all the main histological cell types can easily be identified and then selected for further study. The method is nondestructive and most information can be obtained with the tissue still embedded in wax making it available for follow-up staining. We have found the technique ideally suited to a wide range of biomedical investigations.”*

**PROF. PETER GARDNER, UNIVERSITY OF MANCHESTER**



FTIR imaging of breast tissue microarray (TMA) to spatially identify regions of interest.

(a) H&E visible image of TMA with an expanded view of the two cores used for constructing the histology spectral database.

(b) False color classification image of breast TMA FT-IR chemical image, where green = epithelium, purple = stroma, red = blood, and orange = necrosis. The expanded cores were used for building the histology model. Each core has a diameter of 1 mm and is represented by ca. 26000 IR spectra.

(c) Receiver operator curve (ROC) for the Random Forest classifier output for the independent test data set. Classification results from independent samples test set presented.

Reprinted and adapted with permission from Paul Bassan, Joe Mellor, Jonathan Shapiro, Kaye J. Williams, Michael Lisanti and Peter Gardner, Transmission FT-IR Chemical Imaging on Glass Substrates: Applications in Infrared Spectral Histopathology Anal. Chem. 86(3) (2014) 1648 -1653). Copyright (2016) American Chemical Society.

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