

# Application note Mechanobiology of tumors

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## Discover mechanical forces with the Pavone: a novel label-free biomarker for tumors

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Mechanical properties in the tumor microenvironment play a crucial role in tumor growth, proliferation, and drug resistance. Mechanical changes in the tumoral tissue and its surroundings support tumor progression and metastasis by altering the metabolism and behavior of cancer cells and the associated stromal cells. In this sense, cell mechanics is a potential label-free biomarker for cancer development. Here, we introduce Pavone - a high-throughput mechanical screening platform for thoroughly characterizing a bladder tumor model.



## INTRODUCTION

Tumor development is a complex, dynamic, continuous, and progressive process affected by the mechanical properties of cancer cells and their environment. Throughout tumor formation, the extracellular matrix (ECM) undergoes intricate mechanical alterations that impact and facilitate tumor progression, particularly during invasion and metastasis formation<sup>1,2</sup>. While cancer cells are generally softer than healthy cells, tumors tend to be stiffer and more heterogeneous than surrounding tissues due to matrix stiffening linked to fibrosis<sup>1,3</sup>.

The inherent complexity and variability of tumor growth dynamics and metastasis timing make early cancer detection challenging. Although most cancers' genetic and biochemical drivers are widely recognized, biophysical stimuli also contribute to tumor growth. Therefore, advanced mechanical characterization tools may deepen comprehension of cancer morphology and mechanics and their role in disease progression. However, conventional methods for mechanically characterizing tumors, such as an atomic force microscope (AFM), are complex and time-consuming. They often involve sample preparations that compromise the tumor's native mechanical properties.

In response to the existing gaps in cancer research, we present the Pavone as a novel instrument to mechanically characterize cells, tissues, spheroids, organoids, and biomaterials in a non-destructive manner. This innovative approach can assay the mechanical environments cells experience in health and disease conditions and elucidate the mechanical responses triggered by disturbances with the potential to unlock innovative therapeutic strategies.

## OPTICS11 LIFE NOVEL TECHNOLOGY

Pavone provides a mechanical screening platform for novel research applications, such as disease modeling, drug screening & delivery, regenerative medicine, tissue engineering, and diagnosis of diseases. In particular, it can identify the mechanical difference between healthy and malignant cells and monitor the mechanical environments in cancer progression<sup>4</sup>. By measuring changes in tumor mechanical properties over time, Pavone assesses the effectiveness of anticancer drugs in reducing stiffness, restoring normal tissue function, and potentially overcoming

tumor drug resistance<sup>3</sup>. Additionally, this technology supports physiologically relevant 3D *in vitro* models, such as scaffold- or hydrogel-based structures<sup>2</sup>, spheroids<sup>5</sup>, and organoids<sup>6</sup>. By creating 3D models of cells and surrounding tissues, researchers recapitulate the complex mechanical and biochemical properties of tumors *in vivo*, optimize therapeutic delivery methods, and discover potential cancer therapies<sup>7</sup>.

This application note describes the mechanical properties of a bladder tumor and surrounding areas using Pavone technology.

## MECHANICAL CHARACTERIZATION OF BLADDER TUMOR

Pavone is a powerful tool for characterizing the mechanical fingerprints of bladder tumors. The deposition of ECM components, mainly collagen, reduces tissue compliance in bladder tumors. Therefore, mechanical testing and characterization of ECM properties in bladder tumors provide valuable insights into the development and progression of the disease and potential therapeutic targets<sup>4,8</sup>. Here, we mechanically evaluated an orthotopic rat bladder tumor model using Pavone. This study was conducted in collaboration with Massimo Alfano's research group at Ospedale San Raffaele<sup>†</sup>.

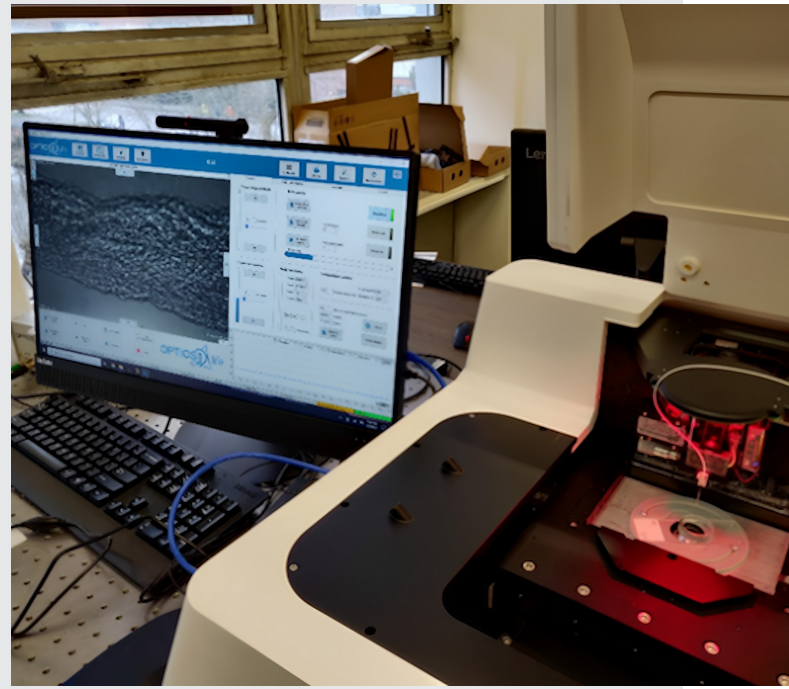
The AY-27 rat bladder transitional cell carcinoma cell line was instilled intravesically into a rat to induce a bladder tumor. After four weeks, the bladder tissue was prepared for histology and mechanical testing. For that, the bladder was instilled with a cryoprotectant (OCT compound) through a catheter before explanting and snap-freezing them. Then, pairs of adjacent 10 and 50  $\mu\text{m}$  thick tissue sections from bladder regions were prepared using a microtome cryostat. The 10  $\mu\text{m}$  thick frozen sections were thawed, formalin-fixed, and hematoxylin-eosin (H&E) stained for histological analyses. For mechanical analysis, 50  $\mu\text{m}$  thick fresh-frozen sections were then thawed in PBS on polarized superfrost glass slides to remove the cryoprotectant prior to mechanical testing. To preserve the hydrated conditions during measuring, plastic rings were attached to the glass slides by applying a small amount of oil to the outer edge (Figure 1). All procedures and studies involving animals were

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performed under protocols approved by the IRCCS Ospedale San Raffaele Animal Care and Use Committee and national and international standard guidelines.

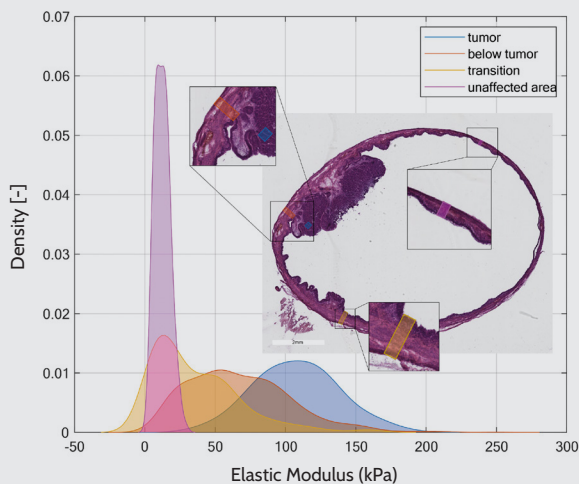
The mechanical properties of bladder tumors were assessed using a probe with a stiffness of 0.5 N/m and a tip radius of 10  $\mu\text{m}$ . The sample was immersed in saline solution during nanoindentation experiments. According to histological analysis, Young's modulus was measured at four locations: tumor, below the tumor, transition zone, and unaffected area.

Our findings revealed high tissue mechanical heterogeneity, in which stiffness correlated with the distance from the tumor area (Figure 2). The tumor area exhibited higher stiffness than the unaffected area. Interestingly, the transition zone between the tumor and the unaffected area underwent mechanical remodeling. Besides, by analyzing two different sites from a single bladder, we confirmed that the pattern was consistent across locations, as shown in Figures 2 and 3.



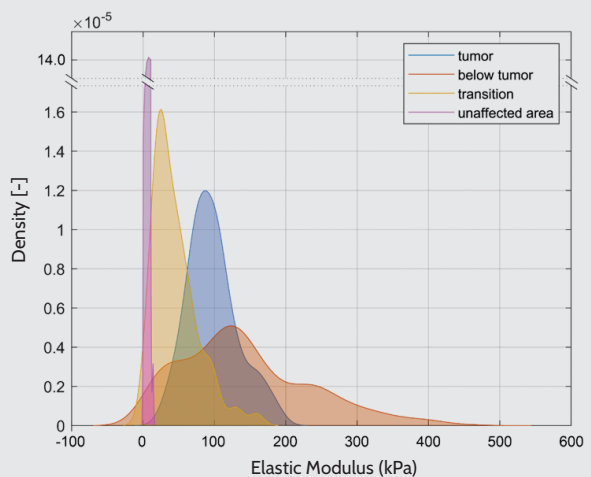
▲ **Figure 1**

Bladder tissue slice placed in the Pavone Nanoindenter. To measure in hydrated conditions, plastic rings were attached to the glass slides by applying a small amount of oil to the outer edge.



▲ **Figure 2**

Mechanical characterization of the bladder. The plot presents the kernel density estimation of Young's modulus for four different bladder regions in an orthotopic rat bladder tumor model: tumor, below the tumor, transition zone, and unaffected area. The corresponding histological figure displays a H&E stained bladder tissue section, highlighting the locations of the four regions mentioned above.



▲ **Figure 3**

Kernel density estimation of Young's modulus for two different sites within a single bladder. The data were obtained from two regions within a tissue slice, with a distance of 300  $\mu\text{m}$  separating them. The distributions of mechanical properties differed, but the stiffness trend was consistent as a function of the location.

## CONCLUSION

Our accurate and non-destructive mechanical assessment method identified that the tumor and the peritumoral region have distinctive mechanical features compared to healthy tissue.

Tumors progressively compromise the mechanical behavior of tissues, making mechanics a potential label-free biomarker for disease development. Understanding the association between mechanical features and clinical manifestations in bladder cancer will contribute to prognostic classification and novel therapies. In light of that, the Pavone is a promising technology for high-throughput cancer research.

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