Optical Flow tracing and Particle Image Velocimetry of micro-contrast agents in micro-ultrasound acquisitions: new method for tumor’s rheological and microenvironment evaluation

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Introduction

Studies of tumor angiogenesis, vascular function and microenvironment are being pursued using multiple approaches. For example, histological and molecular methods readily provide quantitative analyses at tissue, cellular, sub-cellular and molecular levels in both preclinical models and in clinical studies. However, these techniques are not suitable for dynamic or functional studies and are highly invasive. On the other hand, imaging techniques provide non-invasive or minimally invasive dynamic measurements of physiological functions in real-time.

Early evidence of vascular phase is very important into the study of tumor growth, because microcirculation plays an important role in the growth, metastasis, detection, and treatment of tumors.

In addition to the physiological and biochemical changes, tumors generate physical forces during growth and progression; these physical forces can compress blood and lymphatic vessels, reducing perfusion rates and creating hypoxia area.

When exerted directly on cancer cells, they can increase their invasive and metastatic potential. Tumor vessels - while provides energy and furnishes nourishment to the tumor - are usually leaky and tortuous, which further decreases tissues perfusion. Hypo-perfusion and hypoxia contribute to immune-evasion, promote malignant progression and metastasis diffusion, and reduce the efficacy of cancer therapies. In parallel, vessel leakiness together with vessel compression cause a uniformly elevated interstitial fluid pressure that hinders delivery of blood-borne therapeutic agents, lowering the efficacy of chemo- and nano-therapies. Moreover, shear stresses exerted by flowing blood and interstitial fluid modulate the behavior of cancer and a variety of host cells. Knowing these physical forces can improve therapeutic outcomes in many cancers.

The aim of our work is to integrate the imaging methods, as micro-US and PA, with molecular modeling, drug design and post processing with Optical Flow (OF) method to understand the effects and the action mechanisms of diagnostic, therapeutic and theragnostic agents on cells, tissues and microenvironment of cancer.

Material & method

The transport of mass, momentum, and energy in fluid flows is ultimately determined by spatiotemporal distributions of the fluid velocity field. Consequently, a prerequisite for understanding, predicting, and controlling fluid flows is the capability to measure the velocity field with adequate spatial and temporal resolution. Among modern airflow measurement methods, Particle Image Velocimetry (PIV) and Particle Tracking Velocimetry (PTV), as visualized and non-instructive measurement techniques, are playing more important role. The Particle Image Velocimetry is undoubtedly one of the most important technique in Fluid-dynamics since it allows to obtain a direct and instantaneous visualization of the flow field in a non-intrusive way. This innovative technique spreads in a wide number of research fields, from aerodynamics to medicine, from biology to turbulence researches, from aerodynamics to combustion processes.

The term Particle Image Velocimetry (PIV), in the technical world, is used to describe a powerful, automated flow visualization method which quantifies the instantaneous flow velocity field in two
dimensions. PIV gives valuable information on how velocity field changes at a specific measurement plane, at regular time intervals, selected by the operator.

All exams were performed with VELOLAZR system (Fujifilm VisualSonics Inc.) and Integrated US transducer LZ-250 which operates between 1 – 24 MHz, with frame rate of 18 fps; fifteen Balb/C bearing to subcutaneous syngeneic breast cancer (TS/A) were recruited.

We analyzed several aspects of cancer rheology behavior, with the impact of MM1 micro-bubble bolus (Vevo MicroMarker, Untargeted MM1 - with a diameter ranging from 2.3 to 2.9 µm - injected in the tail vein); the acquisitions modality was evaluated with nonlinear contrast agent (NLCA) acquisitions (fig.1a - 1b). The micro-ultrasound contrast agent monitoring is lasted 30 minutes after injection.

**Results**

PIV involves two processes, i.e. capturing the image (with 2D slice-by-slice scanning approach) for the visualization and the images analysis in order obtain 2D and 3D parameters values, as vector flow, velocity (with the vector components), vorticity, shear rate and strain rate data.

The vector plot shows velocity vectors every fourth column, and the background color contour map corresponds to velocity magnitude (fig. 2a)

An instantaneous echo particle image velocimetry vector field is shown in fig.2b, and in fig.2c streamline are represented; a streamline is a curve parallel to the velocity vector. In fully developed flow, streamlines coincide with the paths of the fluid particles.

Sub-volumes with distinct property's inside tumors can now be identified with OF analysis (fig. 3), showing velocities magnitude with its components, shear rate, viscosity, vorticity, strain rate (fig. 4-5) and divergence numerical quantification. In the figure, the different strain rate values and trend in different points of tumor vector map are showed; vessel bifurcation and bending as well as diameter reduction also
produce local vortices with increased mass transfer rates and are therefore considered as potential docking points for circulating cancer cells.

All elaborations were performed with Particle Image Velocimetry in MATLAB platform; code adaptations are introduced to improve the accuracy and applicability of ultrasound PIV.

After PIV elaboration, 3D volume reconstruction with Mathematica 10 code was obtained; we wrote this software section which could evaluate the tridimensional data reconstruction (fig. 6) and assess the histogram components.
In order to obtain a quantification of the rheology parameters correlated with PIV color map, a dominant color count was performed with Mathematica 10 software on 3D reconstruction.

In this case, we show the values obtained for volume of fig. 6.

3D dominant color count:

{{13522}, {17900}, {26342}, {30161}, {38244}, {63541}, {84909},
{87328}, {99585}, {109118}}

- 13522: low velocity into the 3D PIV map reconstruction (fig.6)
- 38244: high velocity value into the 3D PIV map reconstruction (fig.6)

3D dominant color count graphic representation (fig. 7):

**Conclusion**

Multi-parametric imaging has many potential clinical roles; it’s useful for pharmaceutical drug development and for predicting therapeutic efficacy.

For this reason, the goal of our research project is to develop a volumetric strategy for real-time monitoring and characterization of tumor blood flow using microbubble contrast agents and ultrasound (US) imaging for preclinical and clinical use, improving sensitivity and resolution of imaging equipment in multimodal multiscale imaging.

This method gets a complete information on rheological phenomena who are involved in the throughout the tumor during diagnostic and drug administration processing or follow-up controls.

**References**

2. Dirk Michaelis, Bernhard Wieneke; Comparison between Tomographic PIV and Stereo PIV - 14th Int Symp on Applications of Laser Techniques to Fluid Mechanics, Lisbon, Portugal, 07-10 July, 2008


