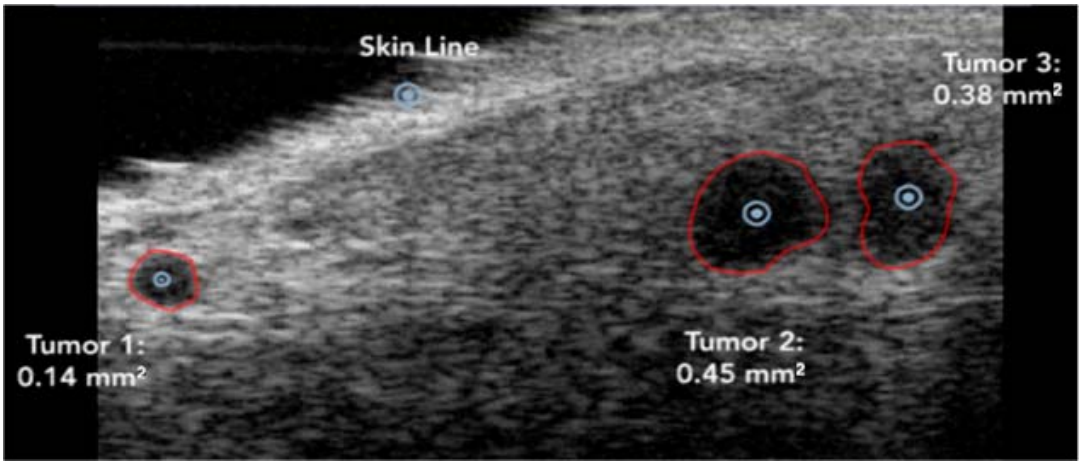
Application Brief – Breast Cancer Research

Executive Summary

Breast cancer research in animal models has long been hindered by the lack of a fast, portable, high resolution, research and animal focused imaging system that can visualize 2D tumor size, 3D tumor volume, neoangiogenesis and blood perfusion *in vivo*, in real-time and most importantly, non-invasively.

In order to ameliorate this problem, VisualSonics has introduced a revolutionary micro-ultrasound system that allows researchers to collect a plethora of important data over the lifespan of animals, thereby significantly reducing the number of animals needed. Furthermore, this system is designed for high-throughput research and is able to image both subcutaneous (xenograft) and orthotopic tumors. 3D tumor volume and tumor vascularity can be quickly quantified, while MicroMarker™ contrast agents allow the quantification of perfusion kinetics and also the possibility to track endothelial cell markers such as VEGFR and integrins.

Hundreds of satisfied cancer researchers using Vevo® micro-ultrasound systems, from institutions such as Vanderbilt, Stanford and Oxford are publishing articles in leading journals such as Science, Clinical Cancer Research, PNAS and Cancer Research; a testament of the power and versatility of high-resolution ultrasound.



Pre-Palpable Tumor Sizing in a Transgenic WAP-TAg Mouse Model,
Image courtesy of Lombardi Cancer Center, Georgetown University.

Background on Breast Cancer Research

Breast cancer is a highly prevalent disease in the female population, affecting 1 in 8 women in the United States.[1] It is no wonder that breast cancer is currently one of the key focuses in biomedical research. Over the past few decades, breast cancer incidence and mortality have decreased steadily, due in part to chemotherapy such as tamoxifen and novel biologics such as Herceptin and Avastin.

Although there have been numerous small triumphs over breast cancer, much work still needs to be done. There were about 40,000 deaths in 2009 due to breast cancer in the United States alone.[1] Small animal research in solid or metastatic breast cancer is a key part of the battle against this disease. In order to facilitate this research, *in vivo* imaging methods are needed to track disease progression in cancer models over a period of time. This offers significant advantages over traditional methods of calipers and histology. By using animals as their own controls, not only can researchers save much time, but also fewer mice are needed for statistical significance and results are less affected by confounding factors. Moreover, these imaging modalities allow the study of effects of therapeutic interventions such as change in tumor volume and angiogenesis. However, many conventional methods such as micro-MRI or micro-CT have significant drawbacks associated, such as significant costs, radioactivity and slow image acquisition.

In today's research, time is of the essence. To test novel hypotheses and discover new drugs, scientists need access to an imaging modality that will grant them the possibility of quickly visualizing tumor growth and blood flow *in vivo*, non-invasively, and in real-time. This is where the Vevo high-frequency micro-ultrasound comes in.

Micro-Ultrasound in Breast Cancer Research

Micro-ultrasound using high-frequency probes and intravenous contrast agent has been regarded by many of the world's top researchers as an attractive technique for accessing tumor size and angiogenic activity. Most research in this area is done using mice, because of their wide availability, variety of strains and close similarities to human biology. Micro-ultrasound is especially suitable to study mice as they are the perfect size to take advantage of the maximum resolution that Vevo systems offer.

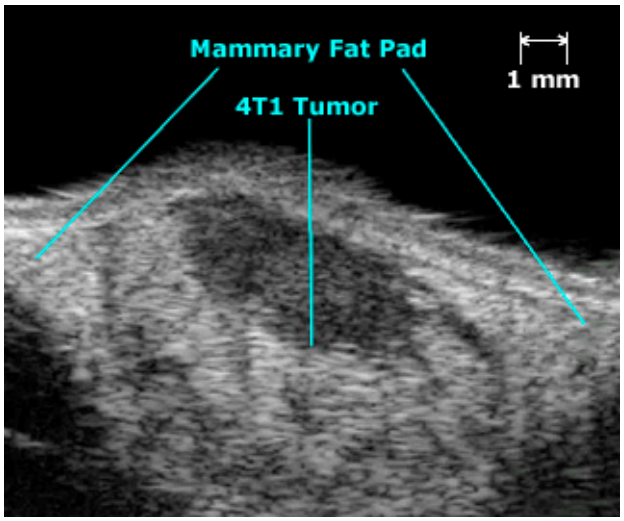
For example, micro-ultrasound has been demonstrated to be a quick and accurate way of tracking tumor sizes non-invasively over a longitudinal study. Dong *et al.* and Wang *et al.* recently published in *Cancer Research* about tracking breast cancer tumor growth *in vivo* with administration of Vitamin E analogues,[2] and a conjugate peptide target of α-tocopheryl succinate (α-TOS).[3] The authors found that these compounds were able to selectively kill angiogenic endothelial cells[2] or induce rapid apoptosis in erbB2-positive breast tumors,[3] respectively. These results were achieved non-invasively using the Vevo 770 system, which quantified tumor sizes accurately and has shown to be superior to digital calipers.[3] Furthermore, percent vascularization of individual tumors were assessed using the Power Doppler Mode, which monitors blood flow in real-time.[2] In conclusion, the authors found that micro-ultrasound imaging allowed both visualization and quantification of non-invasive tracking of tumor size and angiogenesis with mice models.

Lyshchik *et al.* used targeted contrast-enhanced high-frequency micro-ultrasound to monitor *in vivo* VEGFR2 expression on tumor vascular endothelium.[4] The authors used ultrasound microbubbles conjugated with an anti-VEGFR2 monoclonal antibody to examine the vasculature of adult *nu/nu* mice injected subcutaneously with 4T1 and 67NR breast cancer cells.[4] The conclusion was that micro-ultrasound is effective when compared with immunohistochemistry and immunoblotting for quantification of relative expression of VEGFR2 in tumor vasculature and also that micro-ultrasound has significant advantages over micro-MRI and micro-CT for this purpose in terms of portability and ease-of-use; it provides high resolution (30μm) images and can be used with contrast agents that are specifically intravascularly confined, thus minimizing nonspecific signals from extravasated contrast agents.[4]

Loveless *et al.* also examined the use of micro-ultrasound to assess tumor vascularity in mice injected with 67NR breast cancer cells[5] and were able to visualize and quantify microvasculature at the capillary level with high repeatability and minimal background noise.[5] The authors found that

micro-ultrasound not only had the distinct advantages of speed and accessibility, but also created high-resolution 3D images of tumor vessels, ideal for studying of anti-angiogenic activity of therapeutic compounds in preclinical models.[5]

Many other researchers, in recent years, have also been using Vevo systems as an easily accessible, applicable, fast and superior way to image tumor volume and angiogenesis. For an extended list of publications, please email us (info@visualsonics.com) to request a copy of the document titled **Top Cancer Research Papers with the Vevo® Systems.**



4T1 Orthotopic Tumor Model in Mouse with Surrounding Tissue.
Image courtesy of Robarts Research Institute, London, Canada.

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VisualSonics' Value Proposition

Cancer research and tumor imaging has traditionally been associated with modalities such as MRI, CT, PET and SPECT. However, despite their various strengths, many of these systems suffer from significant drawbacks, including significant costs and radiation. Furthermore, none of the above imaging modalities is real-time *per se*, compared with the Vevo system which can capture images instantaneously and construct 3D tumor images in seconds. In fact, it has been demonstrated by Loveless *et al.* that micro-ultrasound produces images eclipsing MRI resolution and that the two imaging systems can be combined to validate each other's results.[6]

Below is a summary of the unique value proposition VisualSonics delivers to researchers with the Vevo micro-ultrasound systems:

1. Non-invasive, *in vivo*, real-time imaging for quick screening (in seconds) and early tumor detection (pre-palpable sizes, $>1x10^{-4}$ mm^3). This allows high-throughput sorting of study animals to optimize homogeneity of test subjects for longitudinal studies.

2. Sophisticated quantification of tumor volumes in 3D. Together with stunning clarity of down to 30µm, the researcher is given maximum flexibility in choosing from a wide variety of tumor models in small animals.

3. Quantification of a plethora of perfusion kinetic parameters associated with angiogenesis and blood flow.

4. Targeted biomarker molecular imaging with antibody-bound contrast agents (VEGFR, integrins, VE-Cadherin, etc.). The unique property of these contrast agents to stay in the vasculature provides unparalleled power for angiogenesis biomarker research.

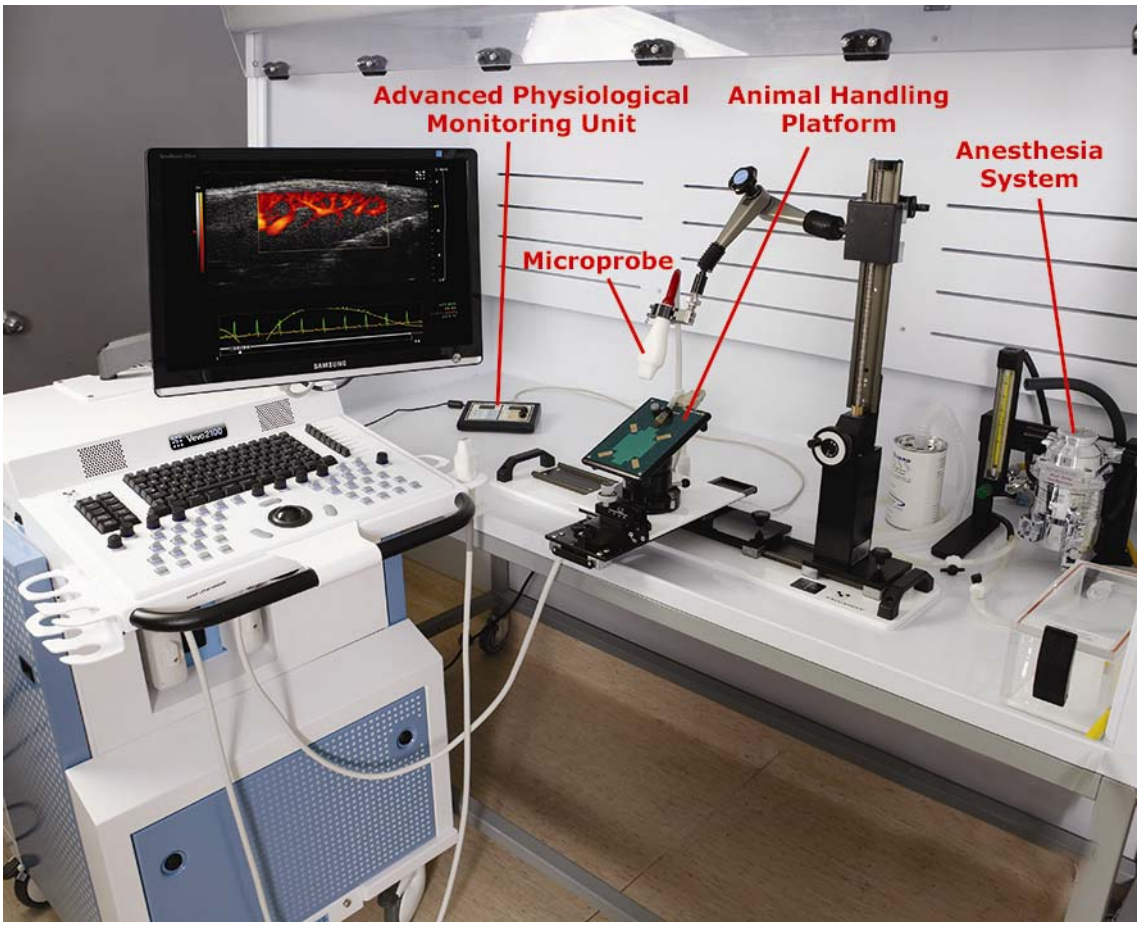
5. Guidance of micro-injections of stem cells, drugs, interstitial pressure probes etc. into tumors or vasculature, without the need for invasive surgery.

6. Detection and quantification of cardiotoxicity in response to cancer therapy. The incomparable temporal resolution and huge variety of software analysis tools allows for elaborate assessment of cardiac function.

7. Dedicated animal platform to monitor ECG, heart rate, body temperature and respiration rates. The researcher is able to keep track of different parameters of the animal physiology in real-time throughout the imaging session and to maintain the animal under ideal

conditions. Together with our specially designed anesthesia system, the animal handling platform allows researchers to spend minimal time on preparatory work and thus optimize throughput.

For a full list of cancer applications with the Vevo, together with examples from literature, please email us (info@visualsonics.com) to request a copy of the document titled **White Paper: High Resolution Micro-Ultrasound for Small Animal Cancer Imaging.**



Vevo® 2100 Micro-Ultrasound Imaging System

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