

High resolution MRI of the mouse mammary gland

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Introduction:

The benefits of breastfeeding on infant health are indisputable and include optimized growth, neurological development and immune function. Additionally, breastfeeding reduces the risk for breast cancer, diabetes and cardiovascular disease¹. To synthesize milk, the resting mammary gland must first proliferate to expand mammary gland mass; it must then differentiate and transition into a secretory organ to synthesize and transfer nutrients during lactation; finally, it undergoes massive apoptosis and autophagy to return to its pre-pregnant state following weaning (involution). Thus, if this process is compromised lactation may be sub-optimal or overtly fail compromising infant health. Moreover, in reproductive age women, the breast undergoes this phenotypic transition with every menstrual cycle. Defects in this process may be associated with benign breast disease (BBD), affecting ~60% of reproductive age women with very few therapies available. In addition, BBD increases the risk for breast cancer by 50%². Therefore, understanding this process has over-arching implications for women's health in general. Our goal was to develop methods to acquire anatomical images of the mouse mammary gland *in vivo* to permit repeated imaging during periods of proliferation, differentiation and regression. These techniques will assist us in developing tools to utilize mouse models to better understand breast development and fibrocystic breast disease in women.

Subjects & Methods:

To explore the MRI capabilities, two mammary glands from wild-type (WT) mice, one nulliparous and one lactating, were dissected and imaged *ex vivo*. The glands were fixed in 4% paraformaldehyde for 24 h and washed/stored in PBS. To reduce the MR imaging time the glands were immersed in a 0.5% Magnevist (Bayer, Germany) solution for at least 3 days before imaging. All MRI experiments were conducted on a Agilent 14 tesla micro imaging system using a home built saddle as well as a home built loop gap resonator. Slightly modified standard three dimensional spin echo imaging (imaging time ~ 30h) yielded a resolution of up to 15 microns isotropic. All data was reconstructed using Matlab (The Mathworks, Inc., Natick, MA). By zero filling the data by a factor of two, the final pixel resolution was 7.5 microns isotropic. Data segmentation was performed using Avizo (VSG3D, USA). To address our primary goal, the mammary gland of a nulliparous WT mouse was imaged *in vivo* using a millipede resonator. A multi slice gradient echo imaging sequence (resolution: 59 x 86 x 300 μm^3) was used to acquire images of the abdominal glands within 3.5 minutes.

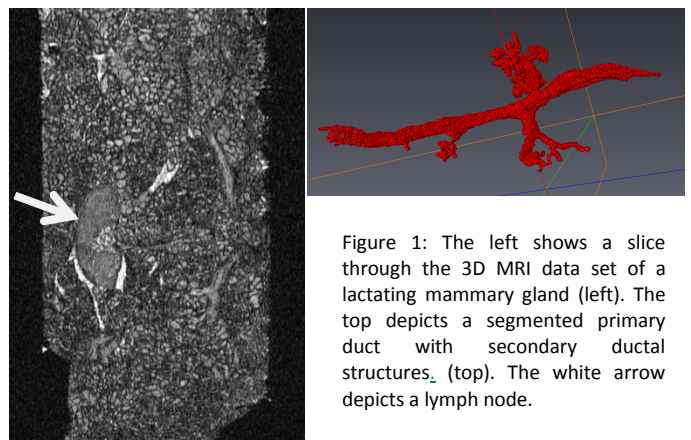


Figure 1: The left shows a slice through the 3D MRI data set of a lactating mammary gland (left). The top depicts a segmented primary duct with secondary ductal structures (top). The white arrow depicts a lymph node.

Results:

The *ex vivo* experiments yielded a very high resolution. We were able to identify the gland structure, the lymph node, and discrete primary ducts and secondary ductal structures in the lactating WT gland as shown in Figure 1. We were able to identify the lymph node as a hallmark of mammary gland position in the *in vivo* experiment (see Figure 2). Unfortunately, we were not able to acquire images of high enough resolution to discern discrete morphological characteristics *in vivo* yet.

Discussion:

MRI provides an excellent tool to study the mammary gland of the mouse *ex vivo* in three dimensions. To determine if we could use MRI to identify defects in mammary gland architecture, glands from transgenic mice with histological defects in mammary gland architecture will be collected, prepared, and imaged accordingly. The *in vivo* results are not satisfying. Although a higher resolution will be possible by using an adapted radio frequency resonator and a longer scan time, it is not clear if the results will be good enough to monitor the changes of the mammary gland through the reproductive cycle *in vivo*.

References:

- [1] do Carmo Franca_Botelho A et al., Asian Pac J Cancer Prev. 2012;13(11):5327-32.
- [2] Miltenburg DM et al., Obstet Gynecol Clin North Am. 2008 Jun;35(2):285-300

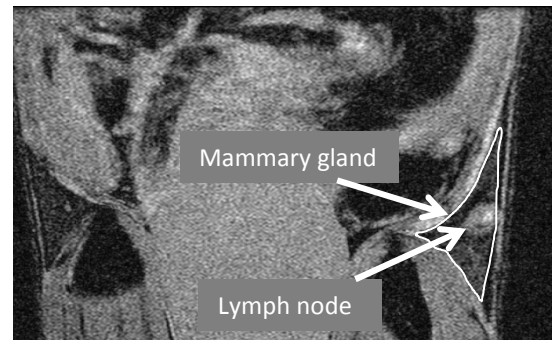


Figure 2: Mammary gland of a virgin WT mouse outlined in white.