Abstract:

The overall aim is to develop an application-specific sample holder and related sample preparation methods for single live cell imaging using a novel high resolution pulse electron spin resonance (ESR) microscope. To fabricate the sample holder, an etched silicon (Si) structure was created using a combination of photolithography followed by deep Si plasma etching. This structure was then bonded with glass cover-slip of 150 µm to form a sealable planar structure. In a controlled atmospheric condition, this fabricated sample holder or, alternatively, phantom structure, is then filled with ESR spin probe solution through inlet-outlet holes on the cover-slip and subsequently sealed by using ultra violet (UV)-curing epoxy. After sealing, the hermetic sample is imaged by means of a spin echo technique using the ACERT (Cornell National Biomedical Center for Advanced ESR Technology) 16GHz 3D Fourier transform (FT) pulsed ESR microscope.

Summary of Research:

Although cylindrical sample tubes are commonly used for conventional ESR and nuclear magnetic resonance (NMR) microscopy, flat sample holders are preferred due to the configuration of the high Q dielectric (SrTiO3) microwave resonators used for ESR microscopy. Doubly stacked ring resonator configurations produce a uniform microwave magnetic field between the resonators where a flat sample holder (~ 0.5 mm thick) can be loaded [1,2]. A flat sample holder configuration also permits us to concurrently study the sample using fluorescence microscopy to obtain complementary information.

For ESR microscopy imaging of biologically-related samples such as tissues or live cells, it is important to prepare the samples using simple, repeatable and reliable techniques. Previous sample holder designs employed glass substrates that were fabricated by wet-etching techniques. However, it is difficult to fabricate well-defined structures in glass due to the isotropic characteristic of the wet-etching process. In order to fabricate well-defined phantom structures and sample holders, Si substrates with clear cover-slip on top are a superior fabrication choice because of the anisotropic etching capability in Si. In evaluation, we have also noted that the high loss factor of the doped Si substrate is problematic; therefore, it is preferable to specify intrinsic Si wafers with high resistivity of > 10,000 Ohm-cm [3].
Figure 1 shows an optical image of the fabricated sample holder with square posts of 100 mm × 100 mm. The sample holder was filled with de-oxygenated 5 mM Trityl radical solution in a controlled atmospheric condition. After sealing, the fabricated sample was then loaded into the pulsed electron spin resonance microscope and imaged, producing the image shown in Figure 2. Nominal resolution of the ESRM image is estimated to be 8 mm × 7 mm × 16 mm in x, y, and z directions, respectively.

Fabricated micro-fluidic channel sample holders of this design will be used for live cell imaging applications.

References:

