Brain tumor surgery aims to maximize tumor resection while avoiding neurological deficits. Accurate characterization of tissue and delineation of resection margins are essential to achieve optimal surgical outcome. Hyperspectral imaging (HSI) and Real-time Rapid Evaporative Ionization Mass Spectrometry (REIMS) are both novel technological adjuncts able to accurately detect intraoperatively zones of low grade glioma without substantially altering the surgical workflow. For HSI, we successfully demonstrate how an HSI system can interface with a clinically approved surgical microscope making use of the microscope’s available light sources and optical ports. We integrated the HSI system into the clinical workflow during brain surgery, specifically for resections of low-grade gliomas (LGG). During the study the acquired dataset was used to train an AI algorithm to detect LGG, providing 81% accuracy on unseen data. Real-time Rapid Evaporative Ionization Mass Spectrometry (REIMS) enables in-situ tissue characterization based on the mass spectrometric analysis of electrosurgical vapors generated during dissection. We demonstrate the use of REIMS for the molecular characterization of brain tumor tissue, thereby providing quasi real-time assessment of tumor type. A classification model containing 31 sampling points in normal brain tissue and 93 in glial tumor tissue provided 89.3% sensitivity and 91.1% specificity. Both techniques, HSI and REIMS display a high intraoperative diagnostic accuracy in terms of glioma tissue detection and are complementary, real-time and label-free methods which we developed and tested up to the stage of high Technology Readiness levels (TRL) of 7 and 5 respectively.