Current knowledge in Alzheimer’s disease (AD) relies to a large extent on fluorescence microscopy of Thioflavin- or immunostained β-amyloid (Aβ) as the traditional hallmark of the disease. However, there is increasing evidence that the pathogenesis of AD is more complex and closely linked to altered lipid homeostasis. This raises the need for a more complete biochemical analysis. In this study, we have complemented 2-photon fluorescence microscopy of Thioflavin-S and Aβ immuno-stained human AD plaques with CARS microscopy. We show that the chemical build-up of AD plaques is more complex and that Aβ staining does not provide the complete picture of the spatial distribution or the molecular composition of AD plaques. CARS images provide important complementary information to that obtained by fluorescence microscopy, motivating a broader introduction of CARS microscopy in the AD research field.