博士論文公聴会の公示（物理学専攻）

学位申請者：Brijesh

論文題目：A Study on Single-cell Analysis by a Stigmatic-type Imaging Mass Spectrometer using Nanoparticle-Assisted Laser Desorption/Ionization Technique
（ナノ粒子支援レーザー脱離・イオン化法を用いた投影型イメージング質量分析装置による一細胞分析に関する研究）

日時：2020 年 2 月 5 日　8:50 ~ 10:20
場所：理学研究科 H 棟　7 階セミナー室（H701 号室）
主査：豊田岐聡
副査：兼松泰男，松野丈夫，上田昌宏（生物科学），久冨修（宇宙地球科学）

論文要旨：

Matrix-Assisted Laser Desorption/Ionization (MALDI) mass spectrometry imaging is a powerful tool to visualize the spatial distribution of biomolecules in biological tissues and cells. However, conventional scanning-type MALDI imaging mass spectrometers do not allow the visualization of biomolecules at a sub-cellular scale spatial resolution because of the dependence of spatial resolution on the laser spot size, which is usually in the range of 10-100 μm. One more issue is the extremely long measurement time. Many researchers have optimized MALDI laser to smaller spot size, but the measurement time becomes longer. To address this problem, a stigmatic-type imaging mass spectrometer equipped with a position- and time-sensitive detector, in which the spatial resolution is not dependent on the laser focus diameter was used in this study. In a stigmatic-type imaging mass spectrometer, the spatial distribution is determined by the combination of the magnification of the ion distribution by the ion optics and the pixel size of the detector. Thus, it has the potential to achieve a high spatial resolution. Furthermore, the measurement time can also be reduced by analyzing a large sample area in a single shot.

Another issue in MALDI is associated with organic matrices. The organic matrices, when deposited on the sample after mixing in a solution, forms large matrix-analyte co-crystals, which reduces the image resolution. Also, organic matrices are easily ionized, so they produce many interference peaks. To address these issues, nanoparticles are used in this study instead of organic matrices.

We analyzed amoeba cells using TiO_2, CeO_2, WO_3, Ag, ZnO, CaO, Fe_2O_3 nanoparticles by a scanning-type MALDI Spiral-TOF mass spectrometer. The metal oxide nanoparticles were deposited on the samples in the wet form after mixing in a solution and, the Ag nanoparticles were deposited in the dry form using an ion sputtering instrument. After the analyses, Fe_2O_3 nanoparticles were found to be the most effective for the analysis of lipids in the amoeba cells, but the quality of the images reduced due to the migration of the analytes in the sample after the deposition of the nanoparticles in wet form.

HeLa cells were analyzed using Ag nanoparticles, which can be deposited in the dry form, by the scanning-type MALDI Spiral-TOF mass spectrometer and, a spatial resolution of 30 μm was achieved. Then, single HeLa cells were analyzed by a stigmatic-type imaging mass spectrometer using Ag nanoparticles and, a spatial resolution of 2 μm was obtained as a result.

The stigmatic-type imaging mass spectrometer equipped with a position- and time-sensitive detector and in combination with nanoparticle-assisted laser desorption/ionization technique showed the potential for high spatial resolution single-cell imaging. Since single-cell analyses are very important for understanding cell metabolism, cellular functions, and disease states, this study will be of great importance for the single-cell biologist and mass spectrometry researchers and inspire them to adopt stigmatic-type imaging mass spectrometer for single-cell analysis.