Introduction

G protein-coupled receptors (GPCRs) are seven-transmembrane receptors and exist on the cell membrane surface in various conditions. It transmits information from the outside to the inside of cells, and controls various cell functions via the G-proteins when combined with specific ligands such as hormones and neurotransmitters. Since they have various bioactivities, GPCRs are the most important targets in drug development. When GPCRs are stimulated by their ligands for a long period, they are internalized from the cell membrane into the cytoplasm as vesicles and form endosomes, with neurokinin-1 receptors (NK1R) causing desensitization (internalization). The granule analysis protocol can be used to analyze GPCR internalization.

Fig. 1: Images were captured using CellVoyager CV6000
(a) Original image of control cells
(b) Original image of substance P (1μM) stimulation
(c) Magnified image from (a)
(d) Magnified image from (b)
(e) Granule recognition of (a)
(f) Granule recognition of (b)
Nuclei is stained with Hoechst33342 and NK1R is stained by Alexa488.
Experiment procedure
1. COS7 strain cells stably over-expressing NK1R were plated on 96-well plates at 10,000 cells/well, cultured for 24 hours, and treated with substance P (0-1 μM) for 20 minutes.
2. The cells were fixed with formaldehyde and stained NK1R with Alexa488, and nuclei with Hoechst33342, respectively.
3. Images were acquired by using the CellVoyager CV6000 under the following conditions:
   - Magnification: 40x objective lens
   - Images captured per well: 9 Fields
   - Z-slices: 15 slices (1 μm step)
   - Exposure time: Alexa 488: 800msec, Hoechst33342: 300msec
4. The images were analyzed by using the “Granule Analysis” protocol.
   - Identify nuclear regions from the nuclear image, and cytoplasmic and granular regions from the NK1R images, respectively.
   - Analyze the association among the regions of nuclei, cytoplasm and granules.

Results and Conclusion
NK1R internalization depending on the substance P concentration was clearly visualized and analyzed by using the CellVoyager and the “Granule Analysis” protocol (Fig. 2). The capability of the “Granular Analysis” software to calculate various numerical data, such as the number, area and brightness of granules, or location and area of cells, enables multilateral discussion. In conclusion, imaging and analysis by using the CellVoyager and “Granular Analysis” protocol will be a powerful tool to discover new drug candidates targeting GPCRs.