

# REBUS ESPER™

## Spatial Omics Platform

### Understanding Cells in Context

To understand biology, you need to understand its most basic unit, the cell.

Information at different scales – from the anatomical to the molecular – must be brought together to understand cells' identities, functions, trajectories and interactions.

High-resolution data with spatial context is key to unlocking new discoveries in neuroscience, cancer, infectious disease, immunology and developmental biology.

### Rebus Esper: Resolution, Scale and Speed

The REBUS ESPER™ is a fully integrated, automated spatial omics platform that delivers quantitative single-molecule, single-cell data with subcellular resolution.

The Rebus Esper enables analysis of cells in their native tissue contexts at high throughput and can run multiple optimized assays.

From discovery to validation to teasing apart the finest details of your hypothesis, the Rebus Esper spatial omics platform can accommodate the right assay at the right time for your research, while always delivering exceptional resolution, scale and speed.

#### High-quality, High-throughput Data

##### Resolution

Analyze biological molecules directly in and across large tissue sections with pristine subcellular detail

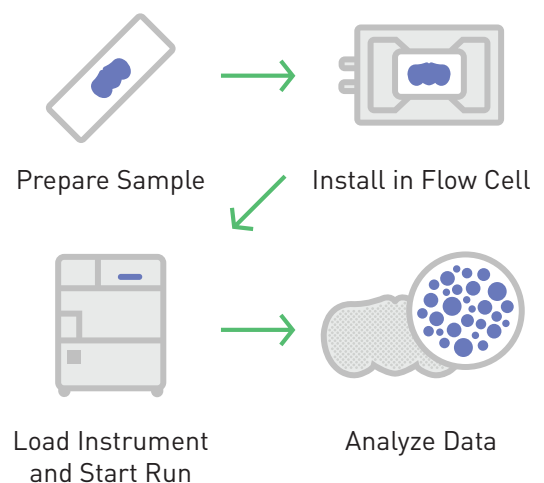
##### Scale

Analyze large single sections or multiple smaller sections in a single run

##### Speed

Tens of millions of data points from hundreds of thousands of cells in less than two days

#### Rebus Esper Workflow



## Single Instrument, Walkaway Operation

Every component of the Rebus Esper spatial omics platform has been developed to work together to deliver high-resolution, high-throughput data while maintaining ease of use and walkaway operation.

Preparing a run follows a simple protocol that requires less than one hour of hands-on time.

After preparing your sample and installing it in the flow cell, load the reagents and flow cell into the instrument. From there, do a quick scan of the tissue to define the area from which to acquire spatial data. Then simply press start to begin the automated run.

Come back after the run is finished to collect processed data, ready for analysis with your software of choice.

## Integrated & Automated Technologies

Advanced imaging, on-system chemistry, and intuitive software have been brought together into a single system, providing a streamlined, end-to-end solution requiring minimal hands-on time.

### Patented Synthetic Aperture Optics (SAO)

In SAO, the sample is illuminated by a series of high-resolution light patterns that are created by the interference of excitation laser beams. A series of low-resolution images is captured by a 20X air objective and automatically reconstructed using proprietary algorithms to generate a single high-resolution image.

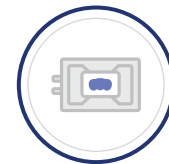
The reconstructed image has the equivalent resolution and sensitivity of an image captured with a high numerical aperture 100X oil immersion lens.

## Rebus Esper Spatial Omics Platform System Components



### Rebus Esper Instrument

Fully integrated system containing all fluidics and imaging components



### Imaging Flow Cell

Receives coverglass with mounted sample; Fluidic ports enable automated reagent delivery and temperature control



### Esper Spatial Studio

Software suite with Esper Control, Esper Process, and Esper Explore software packages



### Workstation

Control Computer running Esper Control software; Data Computer running Esper Process and Esper Explore software packages; Workstation Monitor

## Automated Fluidics for On-System Chemistry

The automated fluidics system handles all sample treatments automatically and works with multiple Rebus Biosystems-developed assays.

All assays are validated and optimized for the Rebus Esper and paired with software that maximizes ease of use.

On-board refrigeration keeps solutions at the right temperature for the duration of experiments. High-speed temperature control within the imaging flow cell allows for the fastest possible reaction times, minimizing the time it takes for you to go from sample to data.

## Intuitive Software

The ESPER™ Spatial Studio software suite includes everything you need to run an experiment, from set up to ready-to-analyze data you can use for single cell analysis and spatial mapping.

Esper Control software guides you through reagent loading, region of interest selection and system control.

Esper Process software processes raw data into high resolution images and uses state-of-the-art computer vision algorithms to detect features, such as RNA spots; segment nuclei based on DAPI; and assign features to nuclei. The final output is a tissue-wide CellxFeature matrix that contains single-cell data for hundreds of thousands of cells and millions of cellular features.

Esper Explore, a visualization package based on the open-source project Napari, allows convenient visualization, exploration and editing of the multi-dimensional data output by the Rebus Esper.

## Technical Specifications

Sample types	Tissue and adherent cells; 12 $\mu\text{m}$ maximum thickness
Lateral resolution	260 nm
Objective lens	Nikon CFI S Plan Fluor ELWD 20xC, 0.45 NA
Camera	Andor sCMOS Zyla 4.2 Plus
SAO illumination laser lines	488 nm (0.5W), 532 nm (2W), 595 nm (2W), 647 nm (2W)
Other illumination	365 nm LED