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# Molecular imaging using laser ablation-postionization mass spectrometry

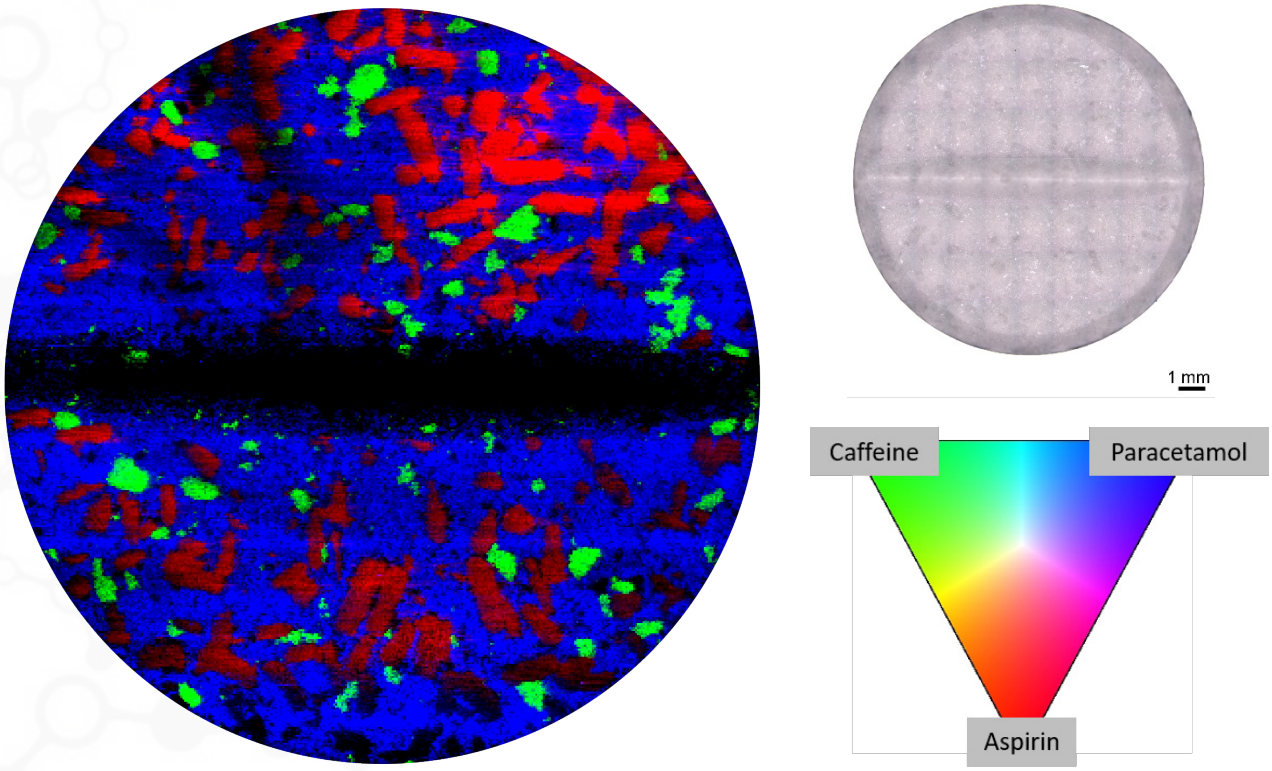


**Figure 1.** RGB image (Left) of aspirin (m/z = 139.03), paracetamol (m/z = 152.06), and caffeine (m/z = 195.08) detected in a Thomapyrin tablet (Top Right). RGB image displayed using ESL's iolite V4 software.

## Brief

Mass spectrometry imaging is a powerful tool that provides spatial distributions of molecules, proteins, lipids, peptides, etc. within a given sample based on molecular mass. Here, a complete solution is presented for converting any mass spectrometer (MS) with an atmospheric pressure inlet (LC-MS) into a laser ablation (LA)-MS for imaging. In this work, an ESL imageBIO LA system is connected to a Bruker micrOTOF MS. The ablated material is ionized using the SICRIT® ionization source from Plasmion and all data is processed using iolite V4.

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# Introduction

Mass spectrometry imaging is a powerful tool that provides spatial information, based on molecular mass, for a given sample. The two main ionization methods in recent years involve laser-based (e.g., LA) or secondary ion-based (e.g., SIMS) techniques. The most common instrumentation for mass spectrometry imaging is MALDI, LA-ESI-MS, or TOF-SIMS. In the case of MALDI or TOF-SIMS, these are commercially available instruments specifically acquired for their capabilities. LA-ESI-MS instruments are not found commercially as a single solution. Therefore, ESL has worked with Plasmion to offer a complete solution that can be added to any LC-MS instrument that uses an atmospheric ionization source (e.g. ESI).

Any laboratory that has an ESI-MS instrument can now couple their MS with our solution. The solution consists of (1) Elemental Scientific Lasers (ESL) imageBIO266 laser ablation system designed for high-speed, high-resolution imaging, (2) Plasmion's SICRIT ionization source, and (3) ESL's iolite version 4 software for data processing.

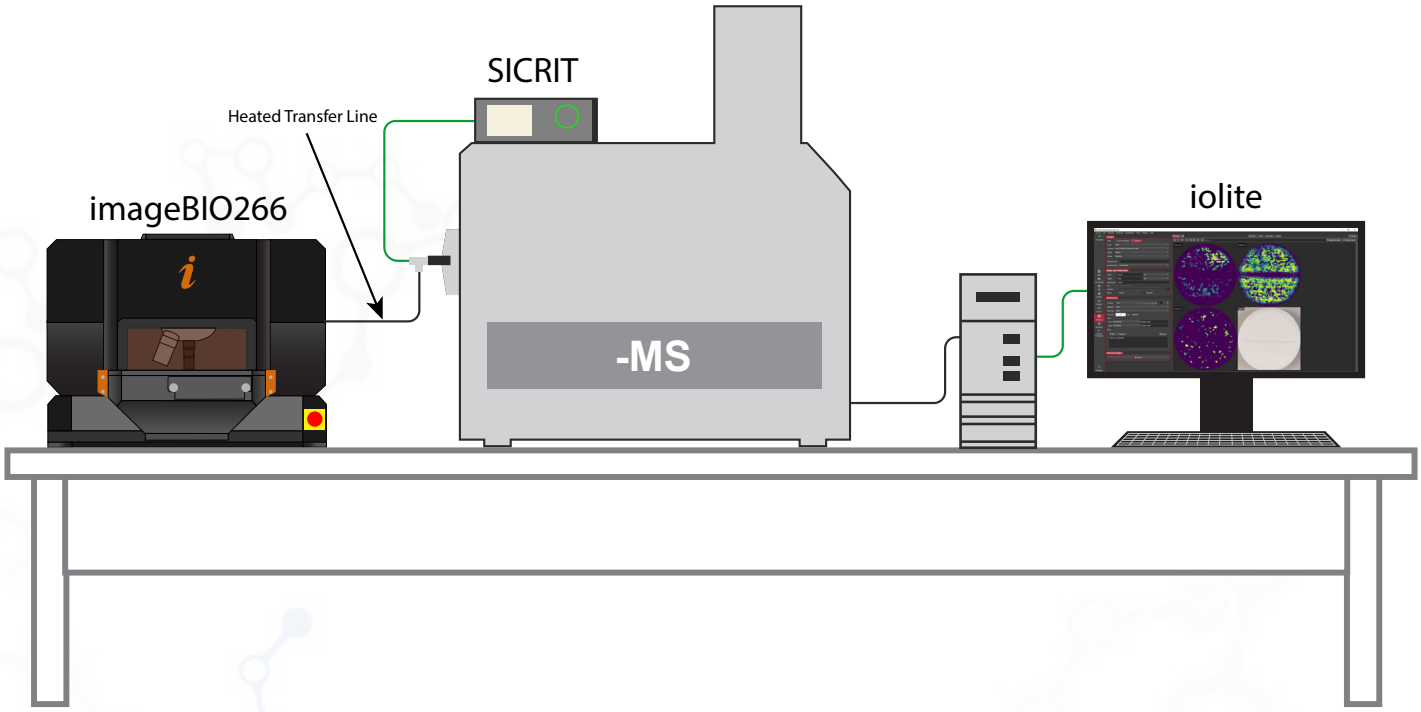


**Figure 2.** Schematic of the complete LA-SICRIT-MS solution.

## Laser Ablation

Laser ablation (LA) is the process of using a nanosecond or femtosecond pulsed laser to remove material one laser pulse at a time from a given substrate. When the laser interaction with the sample occurs, a plasma is formed. Within this plasma, the material is atomized, ionized, and vaporized. When this laser-induced plasma cools, nanoparticles are formed. These particles are then moved from the laser ablation chamber through a heated transfer line and into the ionization source using $N_2$ gas.

The imageBIO266 (Fig. 3) is an advanced state-of-the-art bioimaging system designed for high-speed, high-resolution imaging. It is equipped with a 266 nm nanosecond laser with operation up to 1000 Hz, a two-volume (TV3) LA chamber, high-precision stages, and mass flow controllers. The system is operated using Active View2 software and triggers the MS for analysis.

Other laser systems are available, such as 193 nm or 213 nm nanosecond, 206 nm, 257 nm, or 1028 nm femtosecond, or continuous wave 10.6 µm, although, 266 nm is the most common for organic and biological materials..

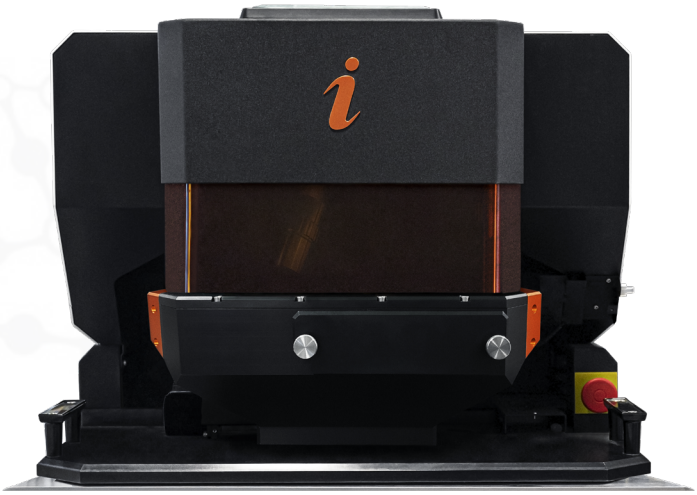

**Figure 3.** Elemental Scientific Lasers' imageBIO266 laser ablation system.

## Ionization Source

For conventional imaging methods such as ESI and APCI, the source in relation to the MS inlet is extremely critical and may lead to tedious and lengthy adjustment/optimization of the source. Plasmion has introduced the soft ionization by chemical reaction in transfer (SICRIT) technology based on dielectric barrier discharge ionization which interfaces directly on the inlet. This creates a cold plasma within the source that all substances pass through, eliminating the critical issue of ion source position and enhances sensitivity.

This innovative technology can be plug and play, interfaced to any MS featuring an atmospheric pressure inlet. The SICRIT SC-30 high-voltage supply powers the ionization source and controls the temperature for the heated transfer line, whereas the source ensures flow through ionization of all compounds drawn into the MS by the inherent vacuum.

**Figure 4.** Plasmion's SICRIT source and SC-30 power supply.

Plasmion
simple smart sensitive

Sicrit
technology

# Data Processing Software

After data collection, all processing is done within iolite V4. Utilizing the laser log (x, y, z stage position per laser pulse, when laser is on/off) and the MS data file, images can be created for each molecular mass of interest. These images can be qualitative or quantitative, depending on if isotopically labelled standards or reference standards were used. Furthermore, images can be overlaid, turned into RGB maps, or visualized as 3D contour plots. Inspection tools allow for easy and quick inspection of the imaged surface (e.g. inspection tool can be used for determining the size of a detected feature or be used to identify the number of spatially resolved features within a given sample).

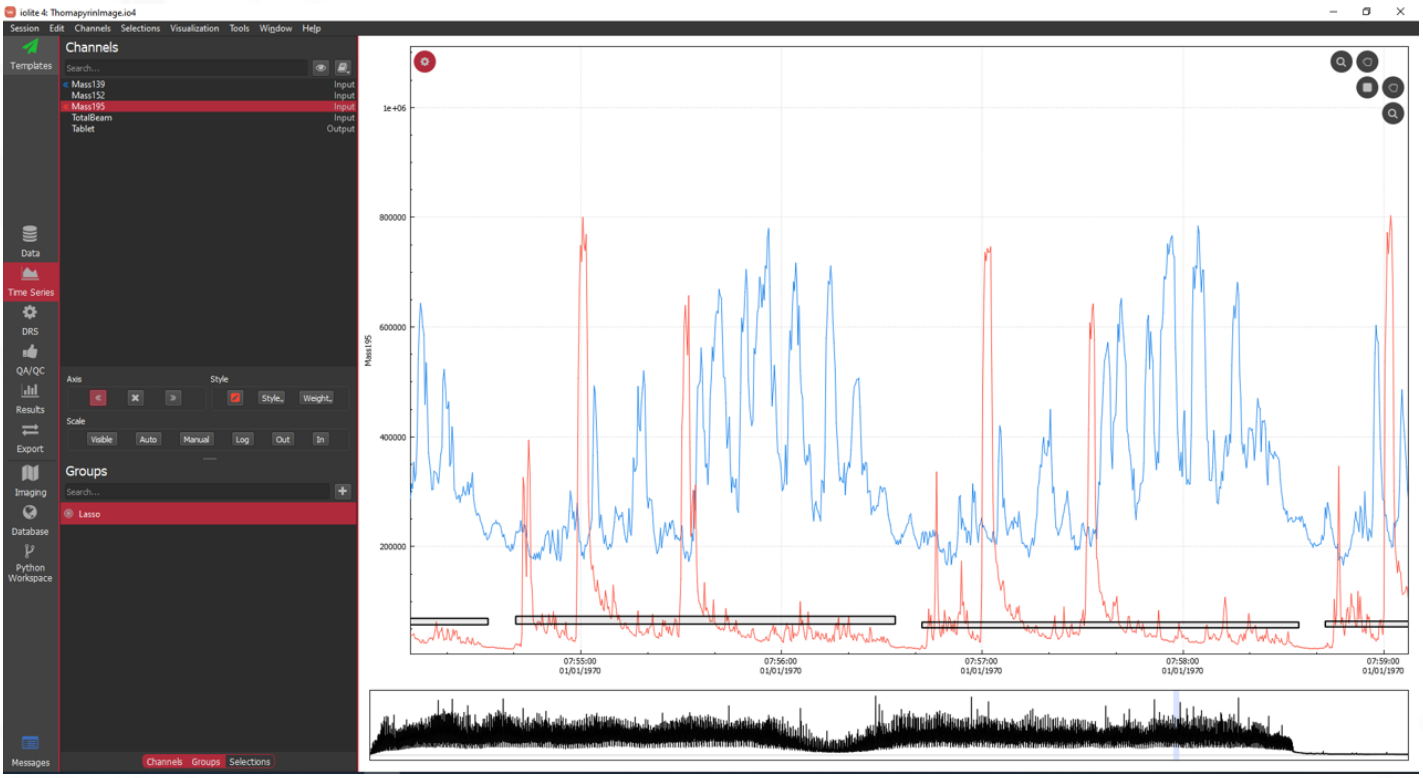


**Figure 5.** Screenshot of the iolite V4 software displaying the time resolved data for m/z = 139 (blue) and 195 (red), along with the laser log laser on information (black). The example here shows two-line scans from the tablet imaging experiment.

# Example Application

To demonstrate the capabilities of this complete solution, a painkiller tablet (Thomapyrin) was analyzed utilizing the imageBIO266, SICRIT source, and a Bruker micrOTOF MS, with data processed in iolite V4. The operating conditions can be found in Table 1. Thomapyrin contains three main ingredients: Aspirin, paracetamol, and caffeine. Paracetamol and caffeine were detected as protonated ions, while aspirin (acetylsalicylic acid) was detected as the protonated fragment ion, salicylic acid. The distribution of these components can be clearly seen in Fig. 6. Various tools are available to inspect the compound distributions, for example, Fig. 7 displays the ability to use the profile tool to determine the particle size from the collected images.

**Table 1.** Instrument settings used in this example application.

| Parameter | Value | Parameter | Value |
|---|---|---|---|
| imageBIO266 | | SICRIT | |
| Laser Wavelength | 266 nm | HV Voltage | 1600 V |
| Laser Spot Size | 30 µm | HV Frequency | 20 kHz |
| Laser Repetition Rate | 10 Hz | micrOTOF | |
| Laser Fluence | 0.25 J/cm$^2$ | Scan Rate | 3 Hz |
| Stage Speed | 90 µm/s | Ion Mode | Positive |
| Gas Flow Rate (N) | 0.7 L/min | | |
| Heated Transfer Line | 300 °C | | |

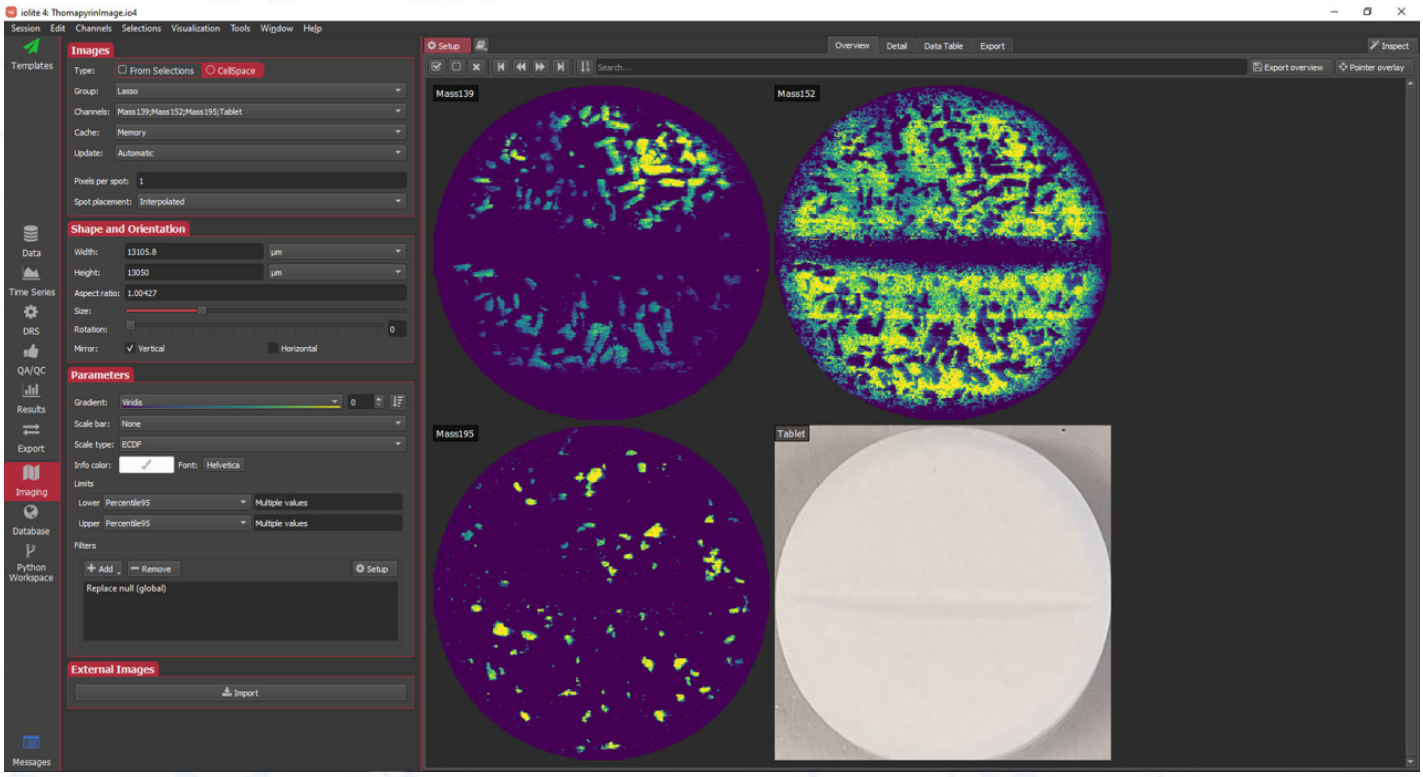


**Figure 6.** Screenshot of the iolite V4 software displaying the distribution of aspirin (m/z 139.03), paracetamol (m/z 152.05), and caffeine (m/z 195.08). The bottom right shows an imported image of the Thomapyrin tablet.

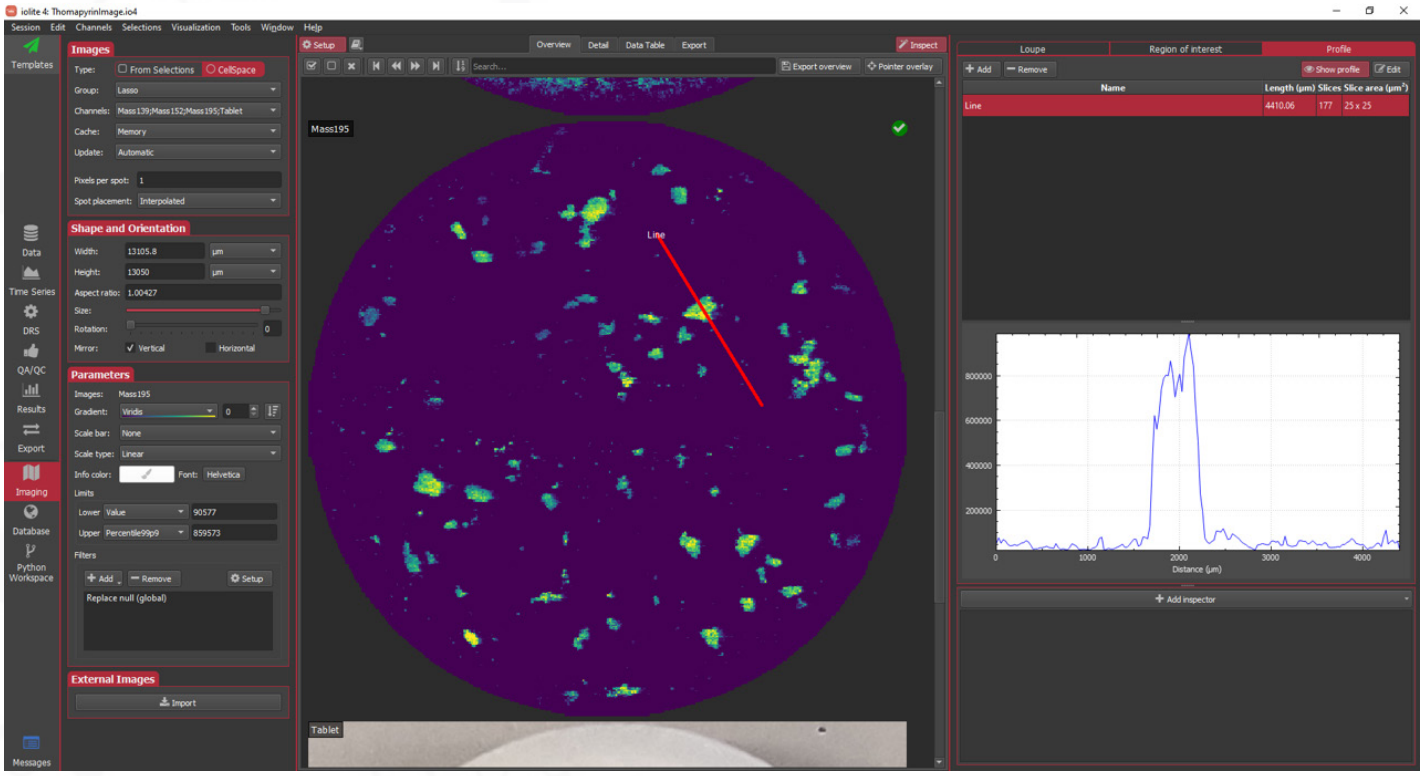


**Figure 7.** Screenshot of the iolite V4 software displaying the distribution of caffeine (m/z 195.08). The inspect tool was selected and used to determine the width of the caffeine distribution in this location. The profile on the right shows the width is ~650 µm x ~850 µm (not shown).

## Summary

The complete solution (laser, ionization source, and software) presented here can be implemented by any laboratory that wants to upgrade their LC-MS, with quadrupole, TOF, orbitrap, or hybrid mass analyzer, to an imaging mass spectrometer. In addition, the SICRIT source provides soft ionization, unique sensitivity, and ionization capabilities covering a broader range of polar and non-polar analytes compared to e.g. ESI or APCI. The minimal void volumes in the source enable faster imaging speeds without "blurring." Iolite software makes data processing seamless and simple for any user but also provides powerful imaging tools to display your results.

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