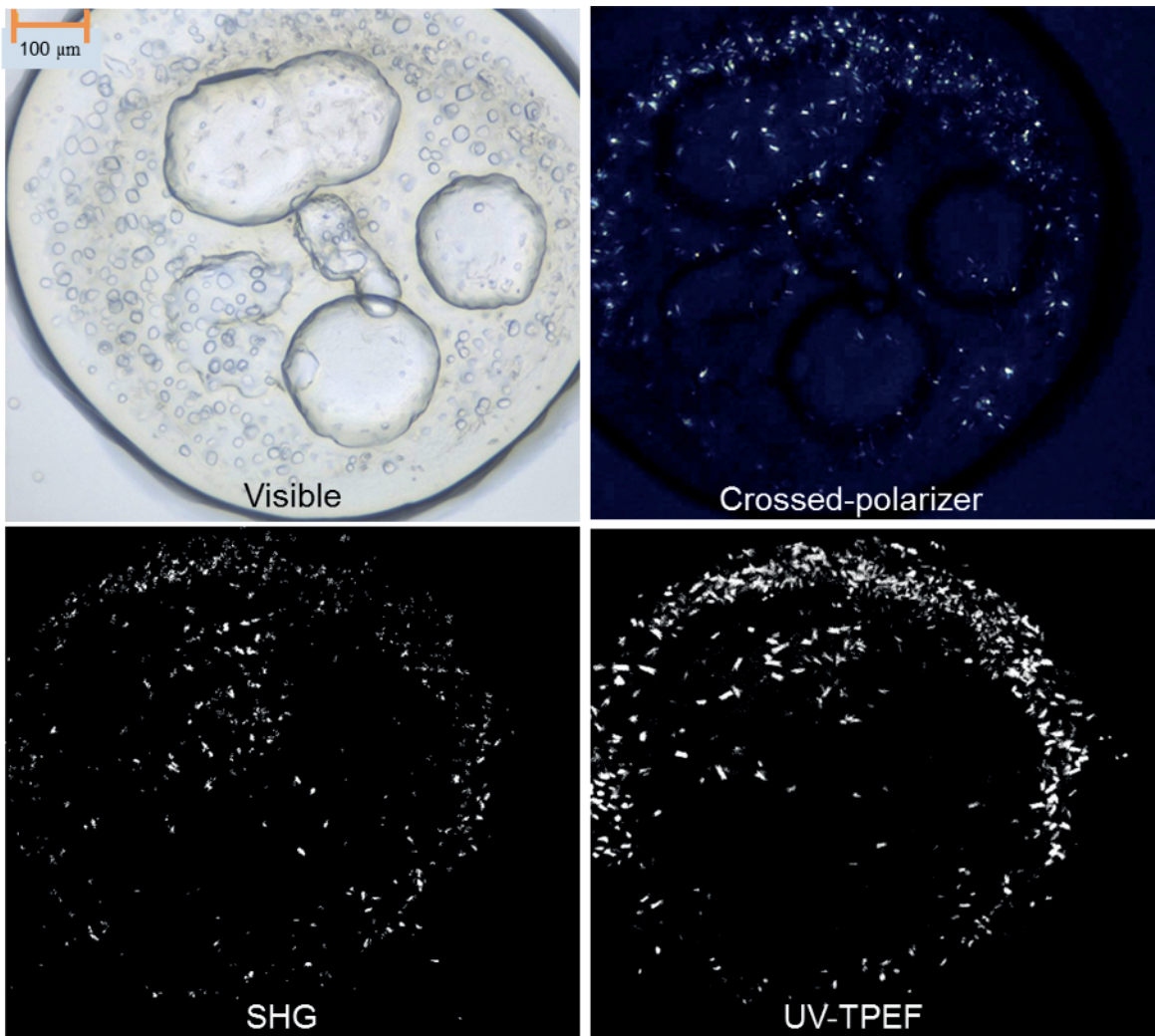# ROCK IMAGER

## User's Guide



Visible

Crossed-polarizer

SHG

UV-TPEF

Original Instructions

Applies to:
RI 1000, RI 182, RI 54, RI 2
FRAP Benchtop & SONICC Benchtop

**FORMULATRIX**

# ROCK IMAGER

## User's Guide

### Original Instructions

Third Edition
Revised January 2016

RIG-V216R116

**⣿FORMULATRIX**

**Formulatrix Product Information**

Formulatrix.com

**Formulatrix Corporate Headquarters**

10 DeAngelo Drive, Bedford, MA 01730  USA  Phone: +1 781 788 0228

For information requests, email info@formulatrix.com. For sales inquiries, email sales@formulatrix.com. For support, email support@formulatrix.com. To comment on Formulatrix documentation, email publications@formulatrix.com.

**Formulatrix European Representative**

Flip Hoedemaeker

Support, Europe

Phone: +31 652 451106

flip@formulatrix.com

# Table of Contents

# Introduction

*Chapter 1*

Thank you for purchasing Rock Imager, an automated imaging system for protein crystallization. This robust, easy-to-use solution incubates[1] and captures superior-quality images of up to 1500 glass sandwich plates or up to 1000 microtiter plates according to a user-defined schedule.

Your Rock Imager must be used in the manner described in this user's guide. Any other use may result in damage to your Rock Imager or personal injury. Formulatrix, Inc. is not responsible in whole or in part for any damages caused by: improper use, unauthorized alterations, adjustments or modifications, failure to comply with the procedures outlined in the Rock Imager User's Guide, or use of the products in violation of applicable laws, rules or regulations. Except as otherwise expressly provided in this user guide, any alteration, adjustment or modification to the products will void the product warranty.

Read, understand and observe all safety information and instructions in this manual before using Rock Imager. Please note specific safety requirements as explained in this user's guide. Failure to follow these instructions could result in serious personal injury.

**Please save this guide for future reference and have it available to all operating personnel.**

Rock Imager is manufactured to the current technical safety-relevant regulations.

---

[1] Rock Imagers 1000, 182 and 54 only

## Symbols and Conventions

| Convention | Description |
|---|---|
| ⚠️ | This icon denotes a caution and advises you of precautions to take. |
| 🚫 | This icon denotes a prohibited action. |
| ❗ | This icon denotes a compulsory action and advises you of actions you must take. |
| ⚡ | This icon denotes a caution relating to electric shock and advises you of precautions to take. |
| 🔥 | This icon denotes a fire hazard, and advises you of precautions to take. |
| ✏️ | This icon denotes a note and alerts you to important information. |
| ☀️ | This icon denotes a laser hazard. |
| ❗ | This icon denotes a warning. |
| **bold** | Bold text denotes items you must select or click in the software, such as menu items and dialog box options. Bold text also denotes parameter names. |
| `monospace` | Text in this font denotes text or characters you should enter from the keyboard. |

# Safety Information
*Chapter 2*

This device contains a laser radiation device within the barcode scanner. Do not stare into the beam or bodily injury may occur.

The door should only be opened under the direction of trained Formulatrix personnel (applies to RI 1000, RI 182, RI 54, SONICC Benchtop, and FRAP Benchtop).

High voltage components in the electronics drawer. Do not disassemble or electric shock may occur.

Only Formulatrix personnel are permitted to transport, assemble, or service Rock Imager.

Use only parts supplied or recommended by Formulatrix in this document or via email.

## Regulatory Compliance

The safety recommendations in this guide are basic guidelines. If the lab where Rock Imager is to be kept has additional safety guidelines they should be followed as well, along with all applicable national and international safety codes.

Formulatrix products are CE (Conformité Européenne) Machinery, Low Voltage, and EMC directive compliant. If necessary, request the official Declaration of Conformity (DOC) from Formulatrix. The product is also CDRH Chapter 21 CFR 1040.10  compliant.

## Equipment Safety Guidelines

- Read all safety and operating instructions before operating Rock Imager.
- Do not place Rock Imager's facilities connections (power and communications cables) where they could cause a safety hazard.
- Do not place the product in a location where it may be subject to physical damage.
- Ensure that all power connections to the product are properly grounded.
- Ensure that Rock Imager receives proper air flow for cooling.
- Do not remove the nameplate, or any warning, hazard, or Equipment Identification labels.
- Turn OFF power before inserting or removing power cables.
- Do not open the door to the Rock Imager chassis without express instruction from trained Formulatrix personnel (applies to RI 1000, 182, 54, and SONICC Benchtop only).

## Mechanical Hazards

Rock Imager is a complex electromechanical device. Only persons with the proper training should service or operate the product.

All facilities to the product must be disconnected before servicing, or injury may result from the automatic operation of the equipment.

## Electrical Hazards

The proper precautions for operating electrical equipment must be observed.

Avoid damage to the power cables. Do not apply unnecessary stress, place heavy objects on or crush the cables or fire may occur.

Switch off all power sources before removing connectors or electric shock may occur.

Arrange cables in accordance with the Technical Standard for Electric Properties and Extension Rules or fire may occur.

Do not use extension cables unless approved by Formulatrix.

## Laser Hazards

Rock Imager robots are sold with laser scanners used to detect barcodes on sample plates.

Laser Scanning devices used in Rock Imager models other than SONICC are CLASS II Laser Products.

Class II lasers are considered safe because of the human eye aversion response, which will limit exposure to no more than 0.25 seconds. Avoid staring into the laser beam.

Lasers used in SONICC imagers are CLASS IV laser products. Do NOT open the door to SONICC Benchtop or Rock Imager 100 Dual Imager - SONICC while the laser is in use under ANY circumstances.

This device contains a laser radiation device within the barcode scanner. Do not stare into the beam or bodily injury may occur.

## Safety Labels

Do not remove or alter any safety labels or warnings from Formulatrix products.

# Specifications

*Chapter 3*

---

## Dimensions

| | | |
|---|---|---|
| **RI 1000** | **Peltier Cooled** | 834 x 2197 x 1085 mm (33" x 87" x 43") 387 kg (864 lbs) |
| **RI 1000** | **Compressor Cooled** | 898 x 2272 x 1117 mm (36" x 90" x 44") 399 kg (880 lbs) |
| **RI 1000** | **Dual Imager** | 834 x 2197 x 1230 mm (33" x 87" x 49") 462 kg (1019 lbs) |
| **RI 182** | | 865 x 1028 x 798 mm (35" x 41" x 31") 161 kg (355 lbs) |
| **RI 54** | | 755 x 1016 x 641 mm (30" x 41" x 26") 114 kg (252 lbs) |
| **RI 2** | | 419 x 580 x 354 mm (16.5" x 22.8" x 13.9") 23 kg (51 lbs) |
| **SONICC Benchtop** | | 642 x 980 x 700 mm (25" x 39" x 28") ~68kg (~150 lbs) |
| **FRAP Benchtop** | | 651 x 877 x 697 mm (26" x 35" x 27") ~45 kg (~100 lbs |

---

## Environmental Requirements

| | |
|---|---|
| **Ambient Temperature Range** | 0° C to 30° C. |
| **Operating Humidity** | 0 - 80% non-condensing. |
| **Storage Humidity** | 0 - 80% non-condensing. |
| **Ventilation** | Do not block the fan vents on the Rock Imager chassis. Maintain at least 2 inches (50.8 mm) of free space between the vents and any obstruction or surface. |

At higher temperatures and humidity levels (e.g.  >25°C and >65% humidity), condensation may form on the outside of the Compressor-cooled RI 1000 when operated at or below 4° C. The system will still maintain the desired temperature in these environments. However, the compressor-cooled RI 1000 operates best in a temperature-controlled laboratory.

> **!** Store Rock Imager within the specified ranges of temperature and humidity where it is not exposed to direct sunlight.

---

## Computer Requirements

**Rock Imager 1000**

- SQL Server 2008 Workgroup Edition (or higher)

- Dedicated database server (Recommended)

- Network connection

- Remote access (e.g. WebEx, gotomypc.com, logmein.com, VPN) (Recommended)

- Email Server access (SMTP server credentials)

- Optional: UPS unit

- Windows XP, Windows Vista 32-bit, or Windows 7 32-bit

**Rock Imager 182/54**

- SQL Server 2008 Workgroup Edition (or higher).

- Dedicated database server (Recommended)

- Network connection

- Remote access  (e.g. WebEx, gotomypc.com, logmein.com, VPN) (Recommended)

- Email Server access (SMTP server credentials)

- Windows XP, Windows Vista 32-bit, or Windows 7 32-bit

- Optional: UPS unit

**Rock Imager 2**

- SQL Server 2008 Workgroup Edition (or higher).

- Remote access (e.g. WebEx, gotomypc.com, logmein.com, VPN) (Recommended)

- Email Server access (SMTP server credentials)

- Network connection (for email access) (Recommended)

- Windows XP, Windows Vista 32-bit, or Windows 7 32-bit

**SONICC and FRAP Benchtop**

- SQL Server 2008 Workgroup Edition (or higher).

- Remote access (e.g. WebEx, gotomypc.com, logmein.com, VPN) (Recommended)

- Email Server access (SMTP server credentials)

- Network connection (for email access) (Recommended)

- Windows XP, Windows Vista 32-bit, or Windows 7 32-bit

- UPS strongly recommended for SONICC

# Electrical Requirements

> ⚠️ Confirm that the power supply requirements are properly met to avoid material damage and fire.

> ❗ The power supply for the system must meet or exceed electrical requirements of the system to avoid damage and risk of fire.

**Rock Imager 1000 – Peltier Cooled System**

- Cooling Unit: 100-240 V, 50-60 Hz, 750 W max, 1 ph

- Robotics: 100-240 V, 50-60 Hz, 240 W max, 1 ph

- Computer: 525 W max, 1 ph

- Recommended UPS: 1500 W

**Rock Imager 1000 – Compressor Cooled System**

- Cooling Unit: 100-240 V, 50-60 Hz, 400 W max, 1 ph

- Robotics: 100-240 V, 50-60 Hz, 240 W max, 1 ph

- Computer: 525 W max, 1 ph

- Compressors: 2x (208-230 V, 50-60 Hz, 2000 W max, 1 ph)

- Recommended UPS: 5000 W

**Rock Imager 1000 – Dual Imager w. Frap (Peltier Cooled)**

- Cooling Unit: 100-240 V, 50-60 Hz, 1000 W max, 1 ph

- Robotics: 100-240 V, 50-60 Hz, 240 W max, 1 ph

- Computer: 525 W max, 1 ph

- Recommended UPS: 3500 W

**Rock Imager 1000 – Dual Imager w. SONICC (Peltier Cooled)**

- Cooling Unit: 100-240 V, 50-60 Hz, 1000 W max, 1 ph

- Robotics: 100-240 V, 50-60 Hz, 835 W max, 1 ph

- Computer: 525 W max, 1 ph

- Recommended UPS: 4500 W

**Rock Imager 182 and Rock Imager 54**

- Imager: 100-240 V, 50-60 Hz, 480 W max, 1 ph

- Computer: 525 W max, 1 ph

**Rock Imager 2**

- Imager: 100-240 V, 50-60 Hz, 150 W max, 1 ph

- Computer: 525 W max, 1 ph

**SONICC Benchtop**

- Imager: 100-240 V, 50-60 Hz, 1195 W max, 1 ph

- Computer: 525 W max, 1 ph
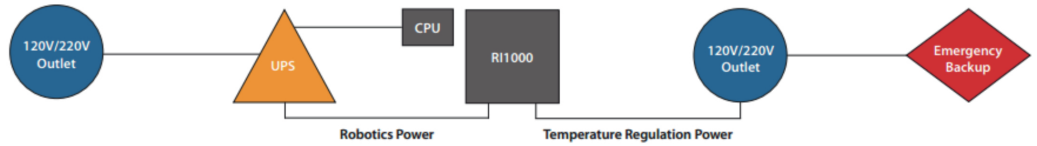
- Recommended UPS: 3340 W

**FRAP Benchtop**

- Imager 100-240 V, 50-60 Hz, 600 W max, 1 ph

- Computer: 525 W max, 1 ph

- Recommended UPS: 2250 W

# Recommended Electrical Configurations

**RI 1000**

**Peltier-cooled RI1000 Recommended Electrical Configuration:**



**Compressor-cooled RI1000 Recommended Electrical Configuration:**
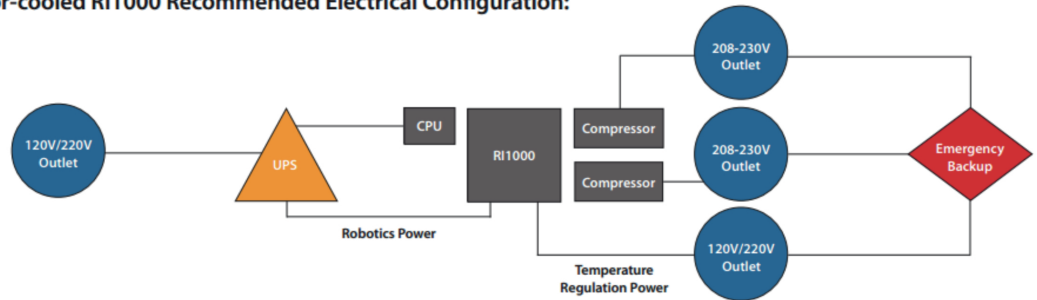


**RI 182, RI 54, RI 2**

# Overview
## *Chapter 4*

This guide presents information about all of the products in the Rock Imager series, including Rock Imager 1000, Rock Imager 182, Rock Imager 54, Rock Imager 2, and benchtop versions of FRAP and SONICC.

## Key Features

- Highest reliability and plate density in the industry with multiple plate types

- Superior optics and intelligent imaging provide industry-leading image quality

- Temperature regulation options for precise temperature control from 30° C down to 4° C

- Seamless integration with Rock Maker or your current crystallization software via Rock Imager API

- Visible light, UV (optional), FRAP (optional), and SONICC (optional) imaging capabilities

- Custom image scheduling

- Nightly diagnostic reports sent to Formulatrix for continued quality control

- Rock Imagers are designed to minimize vibration to microtiter plates to prevent environmental disturbance

- Temperature control with sensors at a variety of locations that monitor temperature to 0.1° C

## Capabilities

**Drop Location.** A proprietary combination of automated lighting and image recognition reliably locates drops in 3 dimensions.

**Auto Focus and Exposure.** Continuous focus algorithm is fast and reliable. Auto focus parameters can be tuned so only the appropriate z-slices are captured. Exposure time is also automatically adjusted to maximize image brightness.

**Region of Interest (ROI).** Specify a region of a drop to zoom into and Rock Imager will automatically capture that area in high resolution.

**Multi-Capture.** Capture multiple images of each drop with user adjustable settings for variables such as: exposure time, polarization state, and condenser aperture value.

**Extended Focus Imaging (EFI).** The highest optical resolution is achieved by increasing the Numerical Aperture (NA) of the objective. A high NA is critical to obtaining images with the contrast and sharpness required to recognize crystal edges. The trade-off, however, is that depth of field (depth of image in focus at one time) is reduced. Combining multiple slices into one EFI image allows the best of both worlds.

**Lipidic Cubic Phase (LCP) Compatible.** All systems can support LCP (or membrane crystallization) imaging with standard microtiter plate heights. The RI 1000 can support up to 1500 individual thin-glass LCP plates with an optional storage rack modification.

**Performance.** Sitting drop imaging as fast as 0.8 seconds per drop with one slice, fixed focus, and fixed exposure.

# RI 1000

The flagship model in the Rock Imager lineup, the Rock Imager 1000 chassis can be configured to store 250, 500, or 1000 microtiter plates, or up to 1500 glass sandwich plates. It is also the only Rock Imager available with compressor-based cooling.

- Up to 1000 SBS plate capacity, or 1500 thin-based LCP plate with optional storage rack modification

- Peltier cooling to 5 °C below or 7 °C above ambient temperature

- Compressor Cooling option available down to 4 °C

- Automated scheduled imaging

- Vibration isolated hotels

- Visible, UV, SONICC/UV-TPEF, and FRAP imaging methods available



**Figure 3-1.** Rock Imager 1000

## RI 182 and RI 54

Rock Imagers 182 and 54 feature many of the same abilities as Rock Imager 1000, although these imagers have limited plate capacity and no compressor-based cooling option.

**RI 182**

- Can hold up to 182 SBS plates or up to 106 Linbro plates

- Capable of Peltier cooling to 5 °C below or 7 °C above ambient

- Performs automatic scheduled imaging

- Vibration isolated storage

- Visible and UV imaging methods available



**Figure 3-2.** Rock Imager 182 / 54

**RI 54**

- Can hold up to 54 SBS plates or up to 28 Linbro plates

- Capable of Peltier cooling to 5 °C below or 7 °C above ambient

- Performs automatic scheduled imaging

- Vibration isolated storage

- Visible and UV imaging methods available

## RI 2

Rock Imager 2 is the smallest of the Rock Imagers, useful for those of you who aren't interested in storage capabilities or who have space concerns but are still interested in the high-powered imaging offered by Rock Imager.

- 2 SBS plate capacity or single Linbro plate capacity

- EasyXtal plate support

- Visible and UV imaging methods available
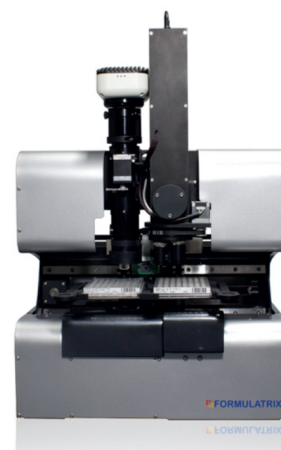


**Figure 3-3.** Rock Imager 2

# User Interfaces

Rock Imager is controlled by the **Rock Imager software**, which enables you to interact with your experiment plates. Your computer screen becomes the microscope's eyepiece and physical optics and imager controls are replaced by on-screen controls.
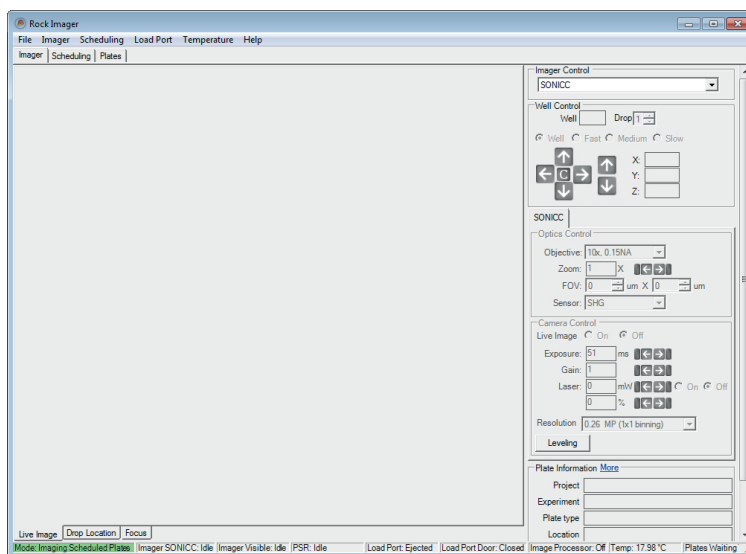


**Figure 3-4.** Rock Imager software

All Rock Imagers integrate with **Rock Maker**, Formulatrix's crystallization experiment lifecycle management software. Automated imaging is scheduled and managed through Rock Maker.
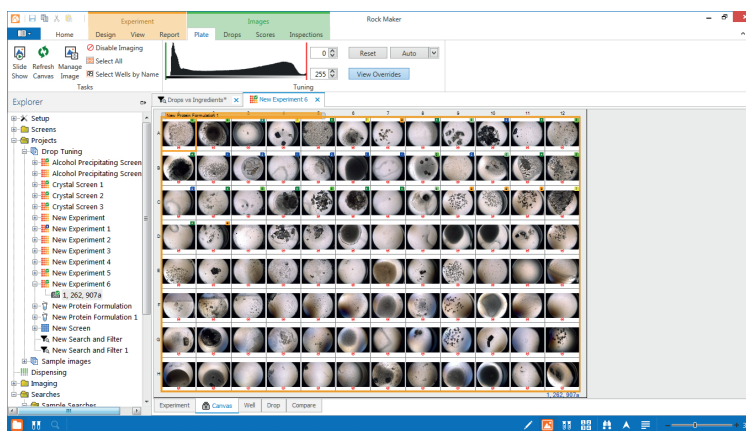


**Figure 3-5.** Rock Maker software

# Imaging Methods

Rock Imager comes standard with a visible light imager. Additional imaging methods, such as UV, FRAP and SONICC can be added at an additional cost.

**Visible Light.** Each visible light imager comes with chromatically corrected optics with a 1-12x continuous zoom option or fixed objectives for the cost-conscious.

**Ultraviolet Light.** UV imaging harnesses the intrinsic florescence of tryptophan under UV light to signify the presence of a protein, reducing false positive and false negative identification of protein crystals. UV imaging is available as a single light path that works in tandem with the visible light imager (best quality), or it can be added to the visible light imaging method in a single light path for a more cost-conscious product.

**FRAP (Fluorescence Recovery After Photobleaching).** FRAP imaging, useful for screening LCP protein crystallization conditions based on membrane diffusion rates, is a process by which a laser bleaches a dye-labeled protein in LCP. The imager then photographs post-bleach activity recording the protein's activity as the bleached dye-labeled protein diffuses out of the LCP and new protein diffuses in. FRAP is available as a separate imaging method in a RI 1000 chassis, or as a standalone benchtop imaging system.

**SONICC (Second Order Nonlinear Imaging of Chiral Crystals).** SONICC imaging detects the second harmonic signal generated by chiral crystals, such as proteins, and is capable of detecting micro crystals (<1 μm) in opaque solutions. It is available as a separate imaging system in a RI 1000 chassis, or as a standalone benchtop imaging system.

**FACIT (Formulatrix Advanced Contrast Imaging Technology).** For those of you in the process of outfitting a new or future lab, a new hardware option is available with visible light imagers. FACIT allows you to control the illumination pattern on a drop in order to see more crystal facets. Any light pattern can be projected onto a drop, including top-left, top-right, bottom-left, bottom-right, darkfield, and brightfield illumination. Illumination patterns are controlled by selecting one of the options form the **Illumination** list in the Rock Imager software.

# Components

*Chapter 5*

## RI 1000

Rock Imager 1000 can include either SONICC or FRAP imaging methods in the base of the chassis, and either a visible light imager or a combined visible and UV imager in the upper portion of the chassis.



Temperature Control

Imager

Plate Load Port

SONICC Imager

Electronics Drawer

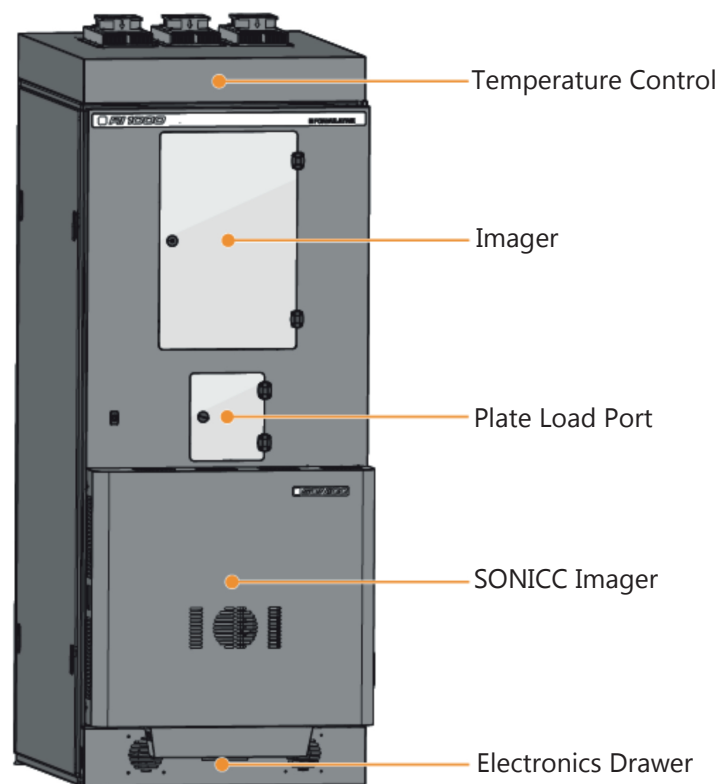**Figure 4-1.** Rock Imager 1000 with SONICC

**Temperature Control.** Rock Imager 1000 monitors and regulates temperatures to within 0.1° C with the assistance of its air circulation system. The base model features a Peltier heat exchanger that regulates temperatures to 5°C below ambient to 7 °C above ambient with 0.5 °C precision. Peltier-enhanced panels can be added to cool the interior to 7 °C below ambient.

A dual compressor redundantly designed system can regulate temperature down to 4 °C with an ambient temperature of 10 °C to 30 °C.

**Load Port.** The load port is where you load and unload plates into the load port hotel. A hotel is composed of slots for several microplates. When you open the door, an internal door closes to protect the internal temperature. You can load entire multi-plate hotels via the load port.

**Sorbothane Mounts.** Sorbothane mounts are used under the robot hands to reduce vibration caused by the robot's moving parts.

**Plate Storage Hotels.** Rock Imager can image and store up to 1000 microtiter plates. This can be reduced to 250 or 500 for cost or use case needs.  For LCP projects, the Rock Maker 1000 hardware can be altered to fit up to 1500 glass sandwich plates.



Sorbothane Mounts for Vibration Isolation

Imager

Plate Storage Hotels

Fault Tolerance with Crash and Plate Presence Sensors

FRAP or SONICC Imager
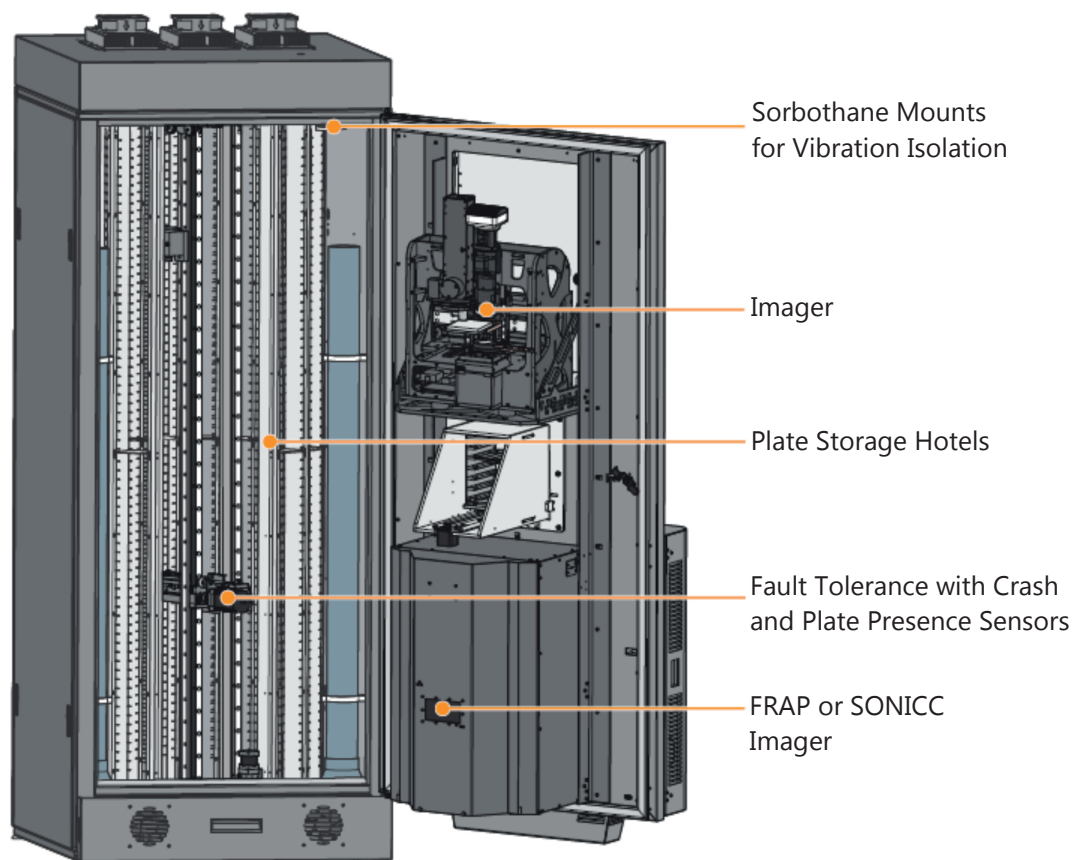
**Figure 4-2.** Rock Imager 1000 Dual Imager Internal View

**Fault Tolerance with Crash Sensors.** Crash sensors detect when a robot arm collides with something during regular operation. In the event of a crash, all operations will cease until a user corrects the problem in order to protect your plates from damage. Usually, plate crashes are caused by unintentional user error, such as leaving a plate lid on a plate.

**Plate Presence Sensor.** The plate presence sensor detects situations where a plate may fall off of a robot hand. This can be caused by inadvertently forgetting to take tape off of a plate. To protect your plates from damage, if an event trips the plate presence sensor, the imager will cease operations until the user addresses the issue.

---

# RI 182 and RI 54

Rock Imagers 182 and 54 share the same style of chassis, only the Rock Imager 182 chassis is deeper to allow for internal plate storage. Additionally, the robot hand in Rock Imager 182 is capable of turning to place and retrieve plates from storage hotels along the inner back wall.

The two chassis, however, share basic external features.



Power Switch

SBS and Linbro
Hotels in Load Port

Load Port Door
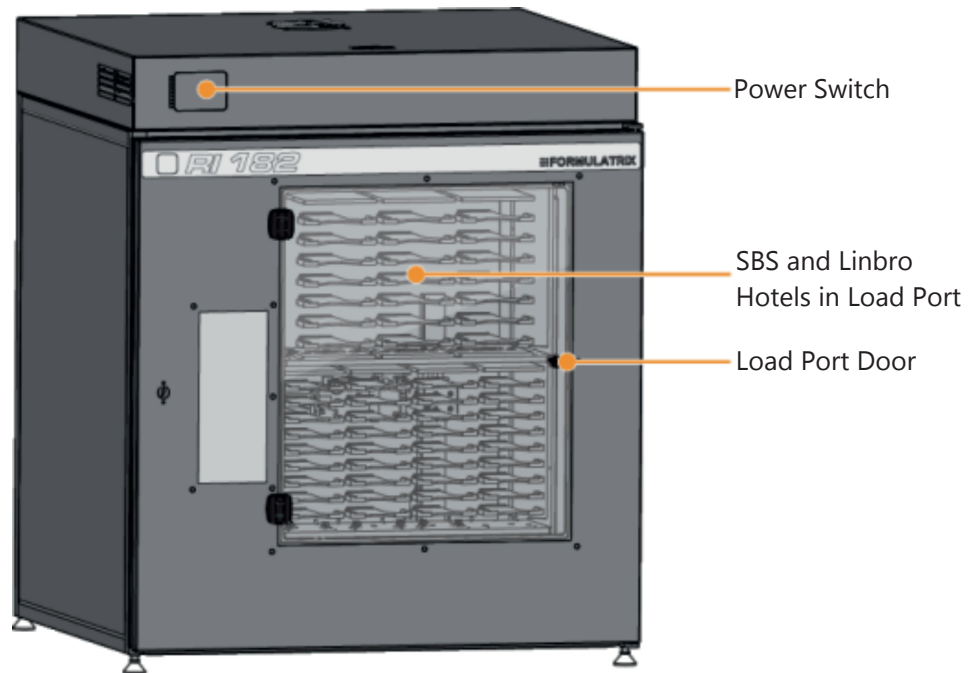
**Figure 4-3.** Rock Imager 182. Rock Imagers 182 and 54 share a  similar chassis design.

**Power Switch.** The power switch is covered by a small door in the upper-left corner of the front of the imager. You should leave the imager powered on during idle times to allow for automatic scheduled imaging and to maintain a constant temperature for any plates stored in the system's hotels.

**SBS and Linbro Hotels in Load Port.** The storage hotels are built to accommodate SBS-footpring plates, but can be altered to accommodate Linbro plates as well.

**Load Port Door.** The most obvious front-facing feature of Rock Imagers 182 and 54 is the clear acrylic door which provides you  with direct access to the storage hotels.

## RI 2

Rock Imager 2 is our benchtop imager designed for visible and UV imaging. It can hold up to two SBS-footprint plates, or one Linbro plate with an optional adaptor. It is sold as either a visible light imager, a visible and UV light imager with separate light paths, or a single light path visible and UV imager. Separate visible and UV light paths provide color visible light images with continuous zoom, while single light path visible/UV imagers provide grayscale visible light images with objectives on a motorized turret.
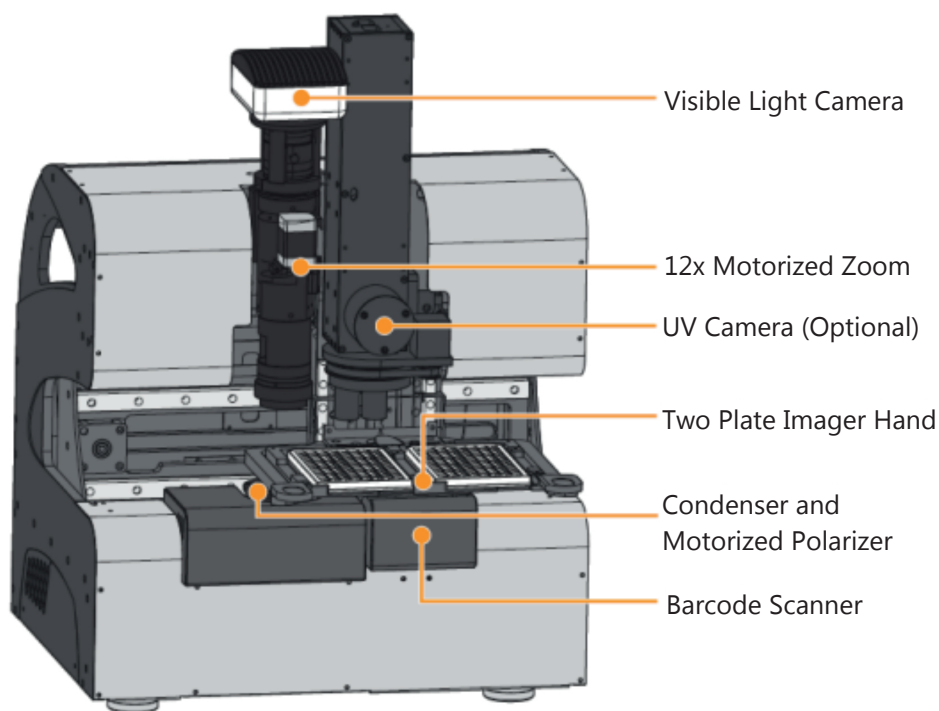


**Figure 4-4.** Rock Imager 2.

**Visible Camera.** All Rock Imagers use a 5-megapixel, 2/3" color CCD camera which captures high-quality images at speeds of up to 25 frames per second.

**12x Motorized Zoom.** 12x motorized zoom optics with <1 µm feature resolution provides sharp, high-contrast images to the camera and allows small feature zooming.

**UV Camera (Optional).** UV Imaging, used to identify protein crystals based on the florescence of tryptophan under UV light, is an optional addition to Rock Imager 2. The UV imager uses 100% UV optimized components including UV grade optics, a UV sensitive camera, and UV lighting, allowing for high contrast images with the ability to see crystals as small as 2 µm. UV imaging can be configured as a fixed zoom objective of 2.5x, 5.0x, or 7.0x, or as a motorized wheel that includes all three objectives.

**Two Plate Imager Hand.** Rock Imager 2 can accommodate up to two SBS-footprint plates. The two plates can be imaged consecutively without user intervention.

**Condenser and Motorized Polarizer.** The polarizer helps to identify crystals based on their birefringence properties, adding color to an otherwise clear object. The condenser concentrates light onto the well being examined in either a cone shape or a column shape, and is used during execution of the drop location algorithm.

**Barcode Scanner.** The barcode scanner allows for automated imaging and passes the images over to Rock Maker to be stored in a user-friendly viewing environment.

# FRAP Benchtop

FRAP (Florescence Recovery After Photobleaching) imaging, useful for screening LCP protein crystallization conditions based on membrane diffusion rates, is a process by which a laser bleaches a dye-labeled protein in LCP. FRAP is available as a separate imager in a RI 100 dual imager or as a benchtop unit, as pictured below.
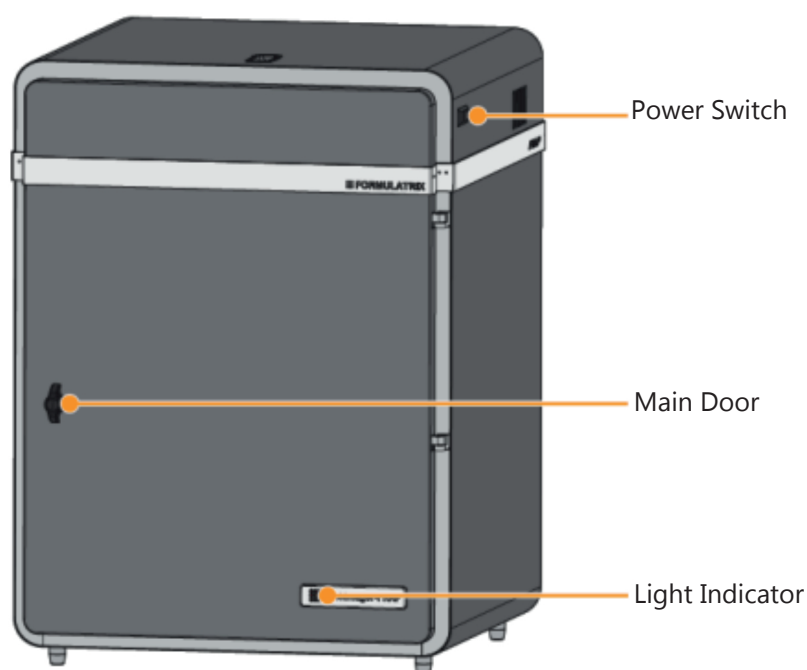


**Figure 4-5.** FRAP Benchtop.

**Power Switch.** A switch that turns the FRAP on and off. FRAP benchtop was not designed to be an incubator, so if not in use FRAP should be powered off.

**Main Door.** The door to FRAP is secured with a hex key locking mechanism. You must open the door to place a plate in or remove a plate from the FRAP benchtop imager. Be sure to close the door securely.

**Light Indicator.** The FRAP barcode lights up during operation to notify you that FRAP imaging is taking place. Do not open the door under any circumstances during FRAP imaging.
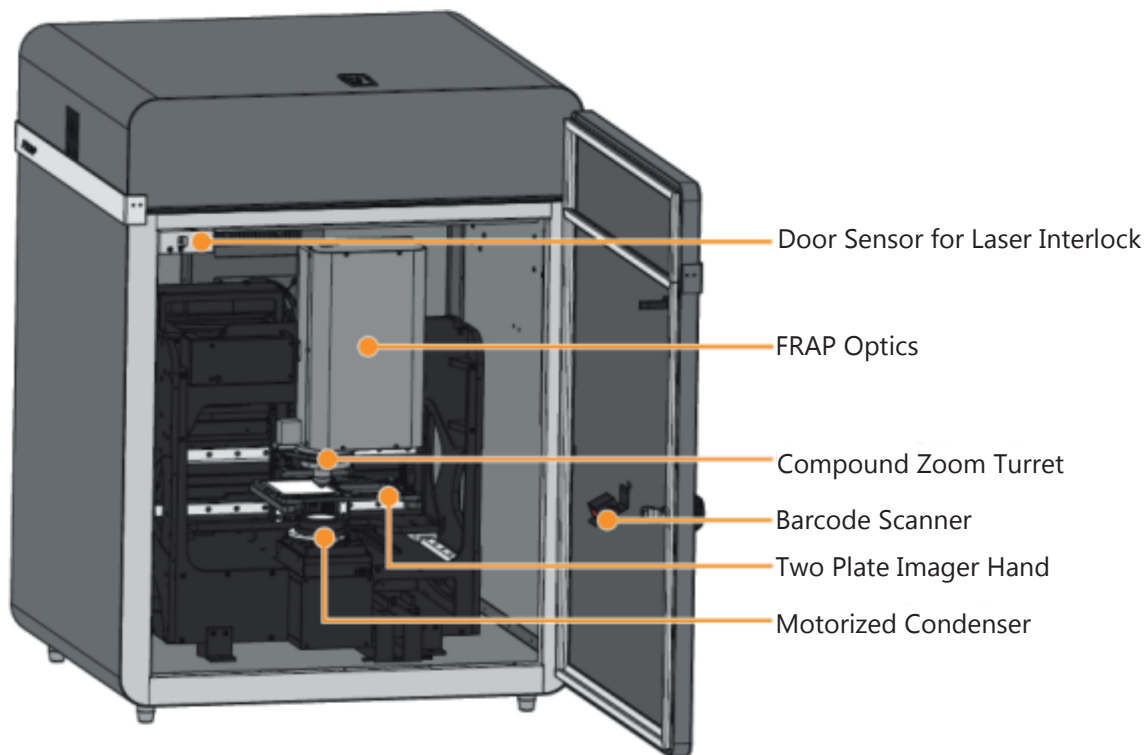
**Figure 4-6.** FRAP Benchtop Internal View.

**Door Sensor for Laser Interlock.** For safety, the laser will automatically turn off when the door is opened; however, imaging will continue and your image results will be affected.

**Barcode Scanner.** Identifies plate information allowing for automated imaging and storing of images for your specific experiment.

**FRAP Optics.** The entire FRAP optics (i.e. bleaching laser, fluorescence light source, and CCD camera) has a footprint of only 15 cm x 27 cm.

**Compound Zoom Turret.** Multiple objectives are available for use providing a large field of view with an option to zoom into areas of interest. FRAP comes standard with two objectives, 1.1x and 5.5x. The lower magnification objective is used for drop location, and the higher magnification objective is use for FRAP experiment imaging.

**Two Plate Imager Hand.** FRAP can accommodate up to two LCP plates that can be analyzed consecutively.

**Motorized Condenser.** The Köhler light source with the motorized condenser provides high contrast images with no sample heating and reliable drop location, which is especially useful for LCP images.

# SONICC Benchtop

SONICC imaging, which includes both SHG and UV-TPEF imaging methods, produces black and white images that makes protein crystal detection easy.

The SONICC Benchtop Rock Imager hardware component guide has also been made into an interactive video. To see it, please point your browser to http://www.formulatrix.com/protein-crystallization/products/sonicc/index.html#tabbed-nav=tab6.



Power Switches

Main Door

Load Port Door
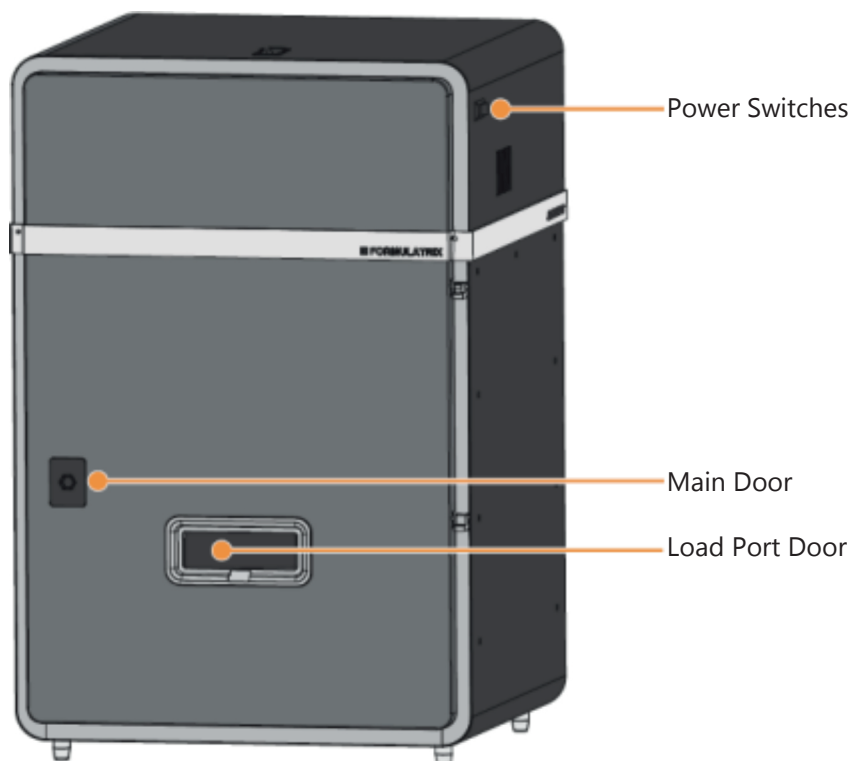
**Figure 4-7.** SONICC Benchtop.

**Power Switches.** SONICC Benchtop has two power switches located on the right side of the chassis, one for the laser and one for the imaging optics. The power switches should be left on at all times, unless you want to power off the imager during periods of inactivity. When you power off the imager, you must first exit the software and then push each power switch to the OFF position.

**Main Door.** The main door is latched securely with a hex key, and should not be opened at any time.

**Load Port Door.** Plates can be safely added to SONICC while the main door is closed and latched by commanding the plate arm to extend out of the door where you can put a plate on the hand.
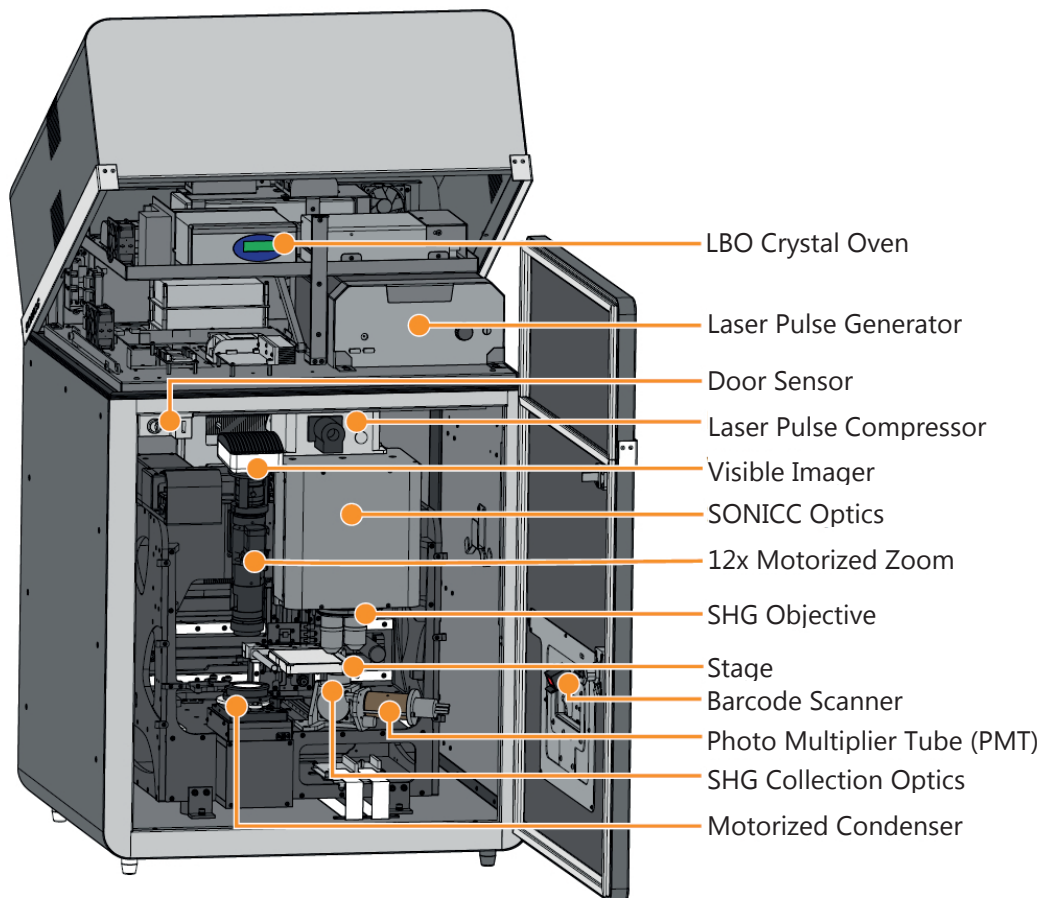
**Figure 4-8.** SONICC Internal View.

**LBO Crystal Oven.** The temperature for the LBO crystal oven is configured during installation and does not need to be adjusted. The LBO crystal oven helps to generate the green femtosecond pulse laser necessary for SONICC imaging (see SONICC on page 29 for additional information about how SONICC imaging works).

**Laser Pulse Generator.** A fiber-based femtosecond laser pulse generator provides a robust laser power source. Laser controls are fully integrated with the Rock Imager software, removing the need for manual adjustments.

**Door Sensor.** The laser will shut off if the door sensor detects that the door is open.  This is implemented as a safety feature. You should not open the door at any time. Continuously opening and closing the door may damage your imager.

**Visible Camera.** The 5 megapixel, 2/3" color CCD camera captures high-quality images at up to 25 frames per second.

**SONICC Optics.** The novel design integrates SHG, UV-TPEF, and laser scanning in an ultra-compact footprint.

**12x Motorized Zoom.** 1-12x continuous zoom allows for drop location with a large field of view (FOV) and then matching magnification for perfect zooming at the drop to fill the FOV.

**Motorized Condenser.** The motorized condenser provides high-contrast images with no sample heating to assist with drop location.

**SHG Objective.** Optional compound zoom available. The high NA objective is used to focus the laser to very small.

**Stage.** The SONICC stage provides precise plate manipulation to within 5 microns. The stage is then be rigidly held  in place so vibration does not interfere with image quality.

**Barcode Scanner.** The barcode scanner identifies plates loaded into SONICC by reading the barcode label and interpreting the label's information.

**PMT (Photo Multiplier Tube).** The photomultiplier tube (PMT) is an extremely sensitive detector of light and is used as the light detector in SONICC. Photons of light generated from the sample are funneled to the PMT creating a cascading electric response that multiplies the signal as many as 100 million times. The PMT response is then mapped to a pixel in an image based on the time that it was acquired to create the SONICC image.

**SHG Collection Optics.** The transmission collection optics collect SHG signal while the epi/backwards collection optics collect UV-TPEF signal. Each path is optimized to only collect the wavelength of interest while minimizing all other signals to create high S/N images.

# Accessories

Accessories are available for purchase online from the Formulatrix accessories store (www.formulatrix.com/store/home.php).

**Load Port Handle**

The load port handle is used with the RI 1000 chassis to add or remove load port hotels from the load port.

To use the load port handle, open the load port door, place the handle into the load port hotel slots and slide the handle upwards until it locks. Carefully remove the load port hotel.

Sliding the handle downwards will disengage the load port handle from the load port hotel.
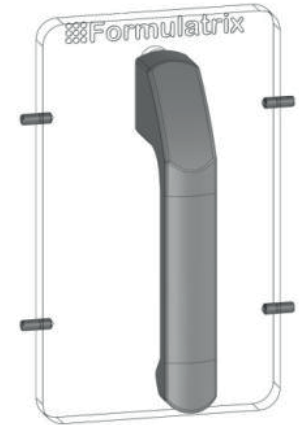
**Figure 4-9.** Load Port Handle.

**L Hex Wrench Key**

The L Hex Wrench key is a special tool used for locking and unlocking the door to the chassis of Rock Imager (except Rock Imager 2, which has no enclosure).

**Figure 4-10.** L Hex Wrench Key.

**SBS and Linbro Load Port Hotels**

Additional load port hotels are available for both SBS (RI 1000, 182 and 54) and/or Linbro plates (RI 182 only).

SBS Load Port Hotel: Part No. 200062.

Linbro Load Port Hotel: Part No. 201237.

RI 1000 SBS Load Port Hotel: Part No. 200709.

**Figure 4-11.** Load Port Hotels.

**Plate Adaptors**

Plate adaptors are available to convert certain plates to SBS footprint plates for use with SBS-compatible Rock Imagers.

Microbatch to SBS Adaptor: Part No. 200936

Vaporbatch to SBS Adaptor: Part No. 200936

**Figure 4-12.** Plate Adaptor.

LCP Plate Adaptor: Part No. LCPPA



**Figure 4-13.** LCP Plate Adaptor.

Linbro Plate Adaptor for RI 2 Systems: Part No. 200547.

EasyXtal Plate Adaptor for RI 2 Systems: Part No. 200544.



**Figure 4-14.** RI 2 Plate Adaptor.

**Barcode Printer Supplies**

4000 Custom Labels for Barcode Printer: Part No. 201621.



**Figure 4-15.** Barcode Labels.

# Imaging Methods

*Chapter 6*

## Visible Light

All Rock Imager models include the visible light imaging method, which features a 5 megapixel, 2/3" color CCD camera capable of capturing high-quality images at up to 25 frames per second.

Your Rock Imager may also be equipped with UV, FRAP (Fluorescence Recovery After Photobleaching), and/or SHG/UV-TPEF imaging methods.

### High Resolution Optics

The 12x motorized zoom allows you to use Rock Imager as a powerful microscope to manually explore the contents of your plates or automate imaging at a precise level of detail. The microscope can resolve features down to 1.6 microns with high numerical aperture objectives providing sharp, high-contrast images.

### Köhler Illumination with Motorized Aperture

Rock Imager uses the same light source as compound microscopes. With Köhler illumination, lenses are used to focus the light on the sample. The motorized aperture in the light path can bend the light from a cone to a column and vice versa for automated contrast adjustment. A cone of light will produce images with the least shadowing, while a column of light produces images with the highest contrast. Adjustments of the angle of the illumination is very important for thin samples where contrast needs to be increased, or for hanging drops where contrast needs to be reduced to decrease shadowing.
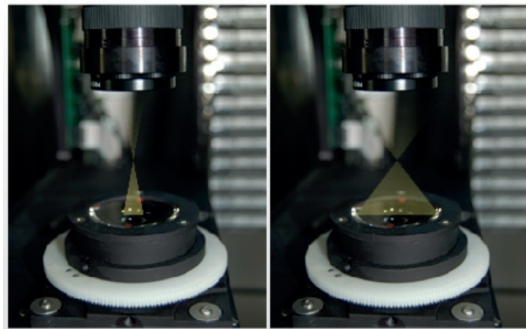


**Figure 5-1.** Kohler Illumination.

**Motorized Polarizer**

To help identify crystals, Rock Imager has a motorized rotating polarizer and a fixed polarizer. The polarizers are 95% efficient and transmit light of 30% at 400-700 nm. The polarizers aid in the identification of crystals by harnessing a crystal's natural birefringence properties, providing a kaleidoscope of colors on the crystal's planes so that you can more easily differentiate a crystal from the plate surface.

## Ultraviolet Light

As the vast majority of proteins contain tryptophan, and tryptophan fluoresces under ultraviolet (UV) light, imaging an experiment plate with UV light photography can help you more quickly differentiate protein crystals from salt crystals. Formulatrix's UV imaging technology uses the fluorescence of tryptophan under UV light to identify whether a crystal contains a protein or is a salt. Our UV solution uses 100% UV-optimized components, including UV-grade optics, a UV-sensitive camera and UV lighting. These are critical to achieving the best image quality and highest imaging speed possible.

Rock Imager UV is available as a retrofit for an existing Rock Imager, or as an option on a new Rock Imager. The UV light path can be built into the visible imager, or added as a separate imaging method (optimal).

**UV Features**

- Distinguish protein crystals from other crystal types

- Find crystals that remain hidden when viewed under visible light

- Available as a standalone inspection station or as a retrofit for an existing Rock Imager 1000.

- Automated switching between UV and visible-light optical paths

- Fully integrates with Rock Maker crystallization software and other Laboratory Information Management System (LIMS) crystallization software

- Compound Zoom Option: Includes a 2.5x, 5.0x, and 7.0x objectives on a motorized turret

- Fixed Zoom Option: Choice of a 2.5x, 5.0x, or 7.0x objective

# SONICC

The Second Order Non-linear Imaging of Chiral Crystals (SONICC) imaging method uses a femtosecond pulsed laser to exploit the frequency-doubling effect found in the vast majority of protein crystals and produces high-contrast images with negligible background signal.

The unique imaging properties of SONICC allow for crystal detection in almost any optical environment including opaque and turbid environments. Only chiral crystals, such as proteins, produce a second harmonic signal and will be detected in the SHG mode. This effect is so precise that SONICC can detect micro crystals (<1 um) and distinguish between crystal showers and amorphous aggregates.

SONICC was invented by Garth Simpson's group at Purdue University and is exclusively licensed by Formulatrix.

**Key Features**

- Quickly find difficult-to-see crystals simply by glancing at thumbnails

- Determines additional optimization conditions

- Detects sub-micron crystals buried in precipitate

- Can distinguish between precipitate and crystals

- Available as an upgrade to Rock Imager 1000 or as a bench top system that includes visible light optics.

**SHG and UV-TPEF**

SONICC has two imaging methods, Second Harmonic Generation (SHG) and Ultraviolet Two-Photon Excited Fluorescence (UV-TPEF). SHG looks for chiral crystals by imaging the sample with IR light, which detects green SHG. UV-TPEF images a sample with a green laser light to try to excite florescence for protein detection. Together these two modes allow you to differentiate aggregates from crystals (SHG mode) and salt from protein (UV-TPEF mode).

The high contrast images obtained with SONICC are due to the specificity to non-centrosymmetric crystals which include chiral crystals. Chiral crystals are those that lack an internal plan of symmetry, and thus its mirror image is non-superimposable on itself. Only those samples that lack inversion symmetry will produce signal. Most salt crystals are symmetric and therefore generate no SHG. Also, all aggregated and solubilized protein will generate no SHG yielding extremely high contrast images.

FRAP, Fluorescence Recovery After Photobleaching, is an optical technique that has been broadly adopted in the bioscience community to quantify two dimension lateral diffusion rates of fluorescently labeled molecules in a thin film layer.

**How Does SONICC Work?**

SONICC is a microscope that uses a laser beam to scan over the sample and then collects the signal generated to create an image.

1. A femtosecond fiber laser is used to generate the 1064 nm incident IR light.

2. The beam is raster-scanned with a resonant mirror (8 kHz) along the fast axis and stepped with a galvo-driven mirror for the slow scan axis.

3. The vibrating beam is expanded and imaged onto the back of the objective.

4. A high N.A. objective is used to focus the incident light onto a sample.

5. The green SHG is collected in the transmission direction and the UV excited fluorescence is detected in the reflected (epi) direction.

6. PMTs detect the SHG via photon counting.

**SONICC Fundamentals**

SONICC relies on the underlying principle of Second Harmonic Generation (SHG), where two low-energy photons combine to form a higher-energy photon under intense electric fields. This process only occurs in noncentrosymmetric ordered crystals. Thus the signal is generated in the presence of chiral crystals with absolutely zero signal occurring from solubilized or aggregated proteins resulting in extremely high contrast images.

Nonlinear effects such as SHG require high electric fields, thus requiring the use of a femtosecond (fs) laser. The laser operates with a pulse width of 200 fs and has high peak powers resulting in nonlinear effects but are short enough to reduce damage associated with localized heating. Further efforts are taken to reduce sample damage by scanning the laser beam quickly so that it does not remain in one spot long enough to heat the sample.

The high contrast images generated by SONICC set it apart from conventional imaging techniques such as fluorescent and birefrigent imaging. Whether probing fluorescent tags or tryptophan, large background signals are generated with fluorescent imaging due to protein in solution or aggregated protein. Incompatible imaging plates that are commonly found with conventional techniques are no longer a problem, as SONICC is compatible with all optically accessible platforms. Fluorescent and birefrigent imaging is also limited to larger crystals (<10 µm) making it difficult to locate those conditions generating crystal showers. SONICC can detect crystals <1 µm allowing for the identification of many more conditions that can be optimized for protein crystal growth.

**UV-TPEF**

Some salt crystals including lithium sulfate and ammonium phosphate form non-centrosymmetric crystals and generate SHG. The use of UV-TPEF can aid in the discrimination between protein and salt crystals.

This imaging mode takes advantage of the intrinsic aromatic amino acid residues (ex. Tryptophan) in the proteins. When excited with UV radiation the tryptophan in the proteins fluoresce making it very easy to determine whether or not a crystal is proteinaceous. In order to probe this fluorescence, the laser is doubled with a NLO (nonlinear optical) crystal from 1064 nm to 532 nm. The green light (532 nm) is then used to image the sample. The two photon equivalent of the green is 266 nm which excites any tryptophan amino acids that are present. The two-photon excited fluorescence (350 – 400 nm) is then collected and used to create a fluorescence image.

It should be noted that it is not necessary for the protein to be crystalline in order to fluoresce. In this way the SHG and UV-TPEF imaging modes act as complements to each other. The SHG channel probes crystallinity and the UV-TPEF channel is specific to proteinaceous samples.

Preliminary experiments show no detectable damage to protein crystals from the laser. In one experiment, a protein crystal was imaged on one half with excessive laser input. X-ray diffraction was obtained from both the exposed and un-exposed halves of the crystals. Both sides diffracted to within expected resolution (~2 A) and within statistical variation (i.e. there was no statistical difference between the diffraction of both sides).

SONICC has also been utilized to image live cells with no observed impact. The cells remained adhered to a Poly-Lysine coated slide before, during and after imaging indicating they remained viable.
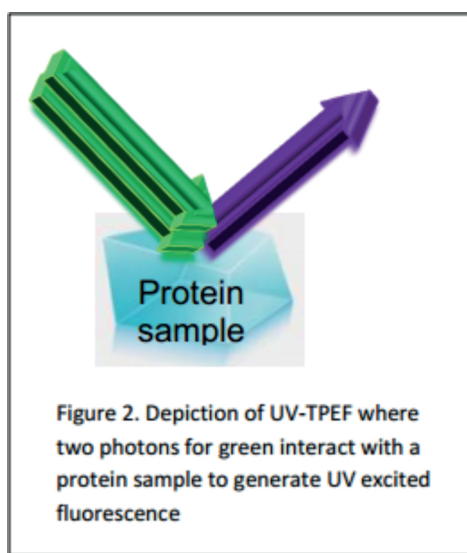


Figure 2. Depiction of UV-TPEF where two photons for green interact with a protein sample to generate UV excited fluorescence

**Figure 5-2.** UV-TPEF Depiction.

# FRAP

FRAP, Fluorescence Recovery After Photobleaching, is an optical technique that has been broadly adopted in the bioscience community to quantify two dimension lateral diffusion rates of fluorescently labeled molecules in a thin film layer.

**How Does FRAP Work?**

1. The LCP-FRAP Imager bleaches the dye-labeled protein in LCP with a laser pulse.

2. A sequence of post-bleaching images are recorded.

3. The image processing function in the imager software (Rock Imager Processor) measures the pixel intensity inside the bleaching spot.

4. Protein mobility parameters are extracted from fitting the pixel intensity recovery curve with either single component or double component 2-D diffusion equation.

# FACIT

FACIT -- Formulatrix Advanced Contrast Imaging Technology -- is a hardware option developed at Formulatrix in which a digital light projector modifies the light pattern used to illuminate your drops. Any pattern you can imagine can be projected onto a drop.

Common patterns from a variety of different angles, including darkfield and glancing illumination, are available to use at a click of a button. Digital light projection means instantaneous illumination pattern switches, allowing for on-the-fly illumination control.



Brightfield          Darkfield

Brightfield          Darkfield          Glancing
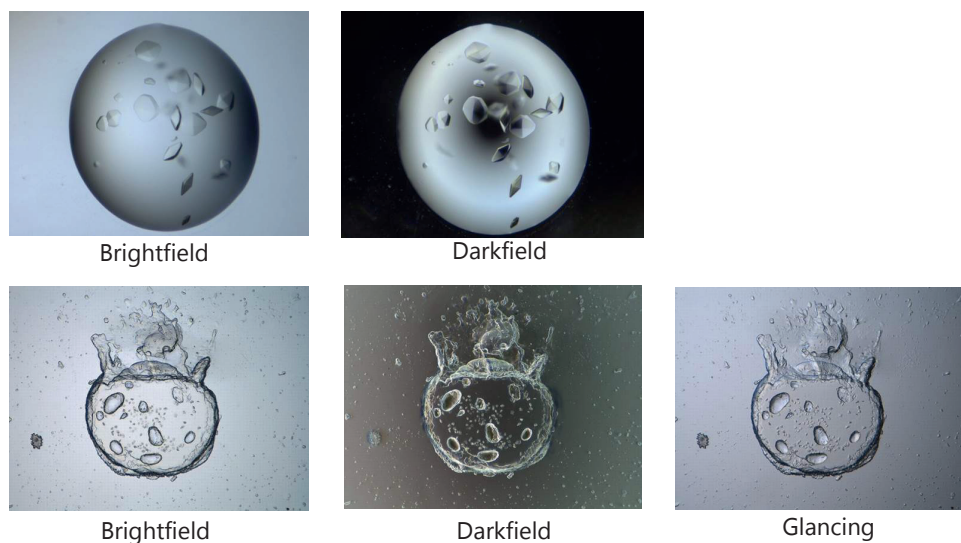
**Figure 5-3.** FACIT illumination patterns

Illumination patterns are controlled by selecting one of the options form the **Illumination** list in the Rock Imager software. FACIT is available in new imagers, it is not available as a retrofit to existing imagers.

# Installation and First Use
*Chapter 7*

The Formulatrix support team will perform the Rock Imager  installation in your laboratory or at your site; do not do this yourself.

You will receive your instrument via a shipping service in a large crate (two extra crates for chillers if you purchase the Rock Imager 4°C).

When you receive your shipping crate:

- Inspect the crate thoroughly for any signs of damage. If you find damage, take pictures and contact Formulatrix and the shipping provider.

- Make sure the ShockWatch sticker is not red (if tripped, the center turns red as shown in the image at right below).



**Figure 6-1.** Shockwatch Labels.

Formulatrix support will have sent you an email with crate dimensions. Please ensure that you have ample space in a dry area away from extreme temperatures to store Rock Imager until support arrives to begin installation. Make sure that the "This Side Up" sticker always points upwards. You will need sturdy moving tools, such as a forklift, if you want to move the shipping crate.

> ⚠️ Only Formulatrix personnel should perform transportation, installation, wiring, inspection, and upgrade procedures, otherwise you may be injured and/or fire may occur.

## First Use

Once Formulatrix technicians have installed Rock Imager, you may follow these steps to turn on and use your device:

1. Locate the power switch.

   - **RI 1000** – The power switches are in the electronics drawer. Open the electronics drawer and turn on the ROBOTICS switch and the COOLING switch.

   - **RI 182/54** – The power switch is in covered by a small door at the front of the device in the upper-left corner.

   - **RI 2** – The power switch is located at the back of the device next to the power cord. For additional help, refer to the Components Section.

   - **FRAP Benchtop** – The power switch is on the right side of the machine at the upper-left corner.

2. **SONICC Benchtop** – The two power switches are on the right side of the machine in the upper-left corner.

3. Start the Rock Imager software by double-clicking the Rock Imager icon  on your computer's desktop.

4. Rock Imager will home the PSR (Plate Storage Retrieval arm), then the Imager hand, and then the Load Port (if you have one).

5. Once Rock Imager is ready, you can begin imaging plates. Refer to the next two sections for more information.

6. When Rock Imager will not be used for an extended period of time, and it is not being used to house experiment plates, you may shut down your imager via the power switch.

> 🚫 Do not operate Rock Imager in an explosive environment, otherwise you may be injured or fire may occur.
>
> 🚫 Never touch moving parts of Rock Imager during operation, otherwise injury may occur.

Rock Imager machines contain barcode scanners that use lasers. Do not stare into the barcode scanner or injury may occur.

If any abnormal operations occur, cease operations immediately and seek technical support from Formulatrix.

# Using RI 1000, RI 182 & RI 54

*Chapter 8*

Rock Imagers 1000, 182 and 54 have the ability to image experiment plates in either Automatic Mode or Manual Mode. In Automatic Mode, a plate is imaged according to the schedule set in Rock Maker. In Manual Mode, you can use Rock Imager's powerful optics as you would a microscope. To switch between modes, use the Scheduling menu.

This section explains how to perform most tasks related to using Rock Imagers 1000, 182 and 54, each of which have the capacity to store plates. If you are looking for instructions relating to manual mode and the manual imaging controls, please see Manual Inspections, Appendix A and Appendix B.

## Adding Plates

**Prerequisites**

- You have created an experiment in Rock Maker. If you aren't interested in tracking conditions with Rock Maker, you can create a proxy experiment in order to expedite the imaging process.
- Your plate has a barcode label affixed to the short side of the plate opposite the A1 position.

**To add a plate to Rock Imager:**

1. Open the door of the load port.

2. Place plates into the load port. Plates should be inserted with the barcode facing away from you. Plates can be placed in any slot, in any order.

3. Close the load port door.

4. Choose from the following options:

   - If you have Rock Imager 1000 or 182, you can move the plate to storage. Moving plates to storage is recommended if you are using Rock Imager 1000 because of greater temperature control. When you move a plate to storage, Rock Imager takes the plate and puts it in a storage hotel away from the load port. The imager will then image the plate according to its automatic imaging schedule.

- To move the plate(s) in the load port to storage, go to the **Load Port** menu and select **Store Contents**. Rock Imager will automatically scan the contents of the load port, and move the plate(s) to storage.

- If you want to manually inspect a plate, first you must tell the imager to scan the barcode: Go to **Load Port**, and select **Scan Load Port**. Locate the Load Port sub-tab (found on the Plates tab) at the bottom-left of your screen. Right-click on the plate of interest and select **Move Plate To**, and then select the imaging method you want to use (e.g. Visible, UV, etc.).

- If you want Rock Imager to image a plate immediately so you can view, score, and annotate images in Rock Maker, first scan the barcode (on the **Load Port** menu, select **Scan Load Port**), then locate your plate on the Load Port sub-tab of the Plates tab. Right-click on the plate and select **Image Plate** (default imaging setting) or **Image Plate Using...** (custom imaging setting). *Note: Rock Imager Processor must be running in order for Rock Maker to receive images.*

## Initiating an Imaging Session

**Prerequisites**

- You have loaded a plate into Rock Imager.

**Imaging**

1. Using the **Search sub-tab** of the **Plates** tab, locate the plate in the imager.

2. Once you locate your plate, you have several options. Click on the plate to highlight it and select from the options below. If you want to image more than one plate, press and hold the `CTRL` key on your keyboard while selecting plates. *Note: All menu options are also available through right-click menus.*

   - To image an entire plate with the default imaging settings, go to the **Imager** menu, point to **Image**, and select **Plate**.

   - To image an entire plate with the imaging setting(s) of choice, go to the **Imager** menu, point to **Image**, and select **Plate Using** Rock Imager will display a dialog box that asks you to select available imaging settings.

   - To manually explore the contents of your plate, conduct a Manual Imaging session (see <u>Manual Inspections</u> on page 53 for more information).

   - To image a specific drop with the default imaging settings, right-click and move the plate to an imager, then go to the **Imager** menu, point to **Image**, and select **Drop**.

   - To image a specific drop with your choice of imaging settings, right-click and move the plate to an imager, then go to the **Imager** menu, point to **Image**, and select **Drop Using**.

- To image tagged drops, right-click and move the plate to an imager, then go to the **Imager** menu, point to **Image**, and select **Interesting Drop(s)**.

- To image tagged drops with a custom imaging setting, right-click and move the plate to an imager, then go to the **Imager** menu, point to **Image**, and select **Interesting Drop(s) Using.**

3. When your plates have been imaged at least once, you can view your images using Rock Maker.

## Searching for Plates in Storage

Once a plate is inside the imager, it could either be in the load port, under the imager, or in a storage hotel (if you have Rock Imager 1000 or 182). If you're not sure where a plate is but you know it's inside Rock Imager, you can use the **Search sub-tab** of the **Plates** tab to locate it.

**Prerequisites**

To locate a plate, you'll need to know at least one of the following parameters:

- Project name

- Experiment name

- Plate number

- Date dispensed

- User name (or owner's name)

- Plate type

- Plate ID

- Barcode

- Location

- State

**To search for a plate:**

1. Go to the **Plates** tab and click on the **Search sub-tab.**

2. Using the first row of the table, start typing your search criteria into the appropriate field on the table. The table filters its contents with each keystroke you make.

3.  Once the plate of interest appears in the table, right-click on the name of the plate to view the options for the plate.

You can:

- See the plate's automatic imaging schedule and other information by selecting **Plate information**.

- Image the plate with its regular imaging setting by selecting **Image Plate**.

- Image the plate with a custom imaging setting by selecting **Image Plate Using**.

- Remove the drop location positions by selecting **Clear Drop Location Positions**.

- Move the plate to the load port, an available imager, or to storage (depending on where the plate is currently located) by selecting the appropriate option after **Move Plate to**.

- Reset your search parameters by selecting **Clear Filter**.

- Save you search results by selecting **Export**.

## Viewing a Plate's Automatic Imaging Schedule

Automatic imaging schedules are set in the Rock Maker software, but you can verify a plate's schedule or view it using the Rock Imager software.

**Prerequisites**

- The plate is inside the imager.

**To view a plate's imaging schedule:**

1.  Use the **Plates** tab to locate your plate inside the imager.

2.  Right-click on the plate and select **Plate Information**. A new window opens that contains two tabs: Schedule and Information. The **Schedule** tab contains the plate's entire automatic imaging schedule.

3.  When you're done viewing the schedule, click **OK** to close the plate information window.

# Removing Plates

When you are no longer interested in imaging a plate, it's time to remove your plate from Rock Imager. In this tutorial, we explain how to safely remove a plate.

> **!** You should never attempt to physically go inside Rock Imager 1000 or 182 to remove a plate from somewhere other than the load port.

**Prerequisites**

- The plate is inside the imager.

**To remove a plate:**

1. If you don't know where your plate is inside the imager, search for your plate to locate it.

    - If the plate is already in the load port, you can skip to step 2.

    - If your plate is somewhere else, right-click the plate, point to **Move Plate To**, and select **Load Port Hotel**.

2. Open the load port door. If you are using Rock Imager 1000, the load port shuttle will move towards you.

3. Remote the plate(s) from the load port.

4. Close the door. If you are using Rock Imager 1000, the load port shuttle will automatically retract.

# Using RI 2, FRAP Benchtop & SONICC Benchtop

*Chapter 9*

FRAP Benchtop, SONICC Benchtop and RI 2 are grouped together because these three imagers are the ones without storage. They also are the three Rock Imagers that can perform Instant Imaging, which allows you to image plates without barcodes.

Like the rest of the Rock Imager line, these three imagers can be used in either Automatic Mode or Manual Mode. In Automatic Mode, plates are imaged according to imaging schedules set in Rock Maker. In Manual Mode, you can use Rock Imager as you would a microscope. By default, these imagers are in manual mode because there is no storage. However, if you want to image the same plate multiple times, you can switch to Automatic Mode.

## Instant Imaging

When you create an Instant Imaging session, Rock Maker creates a special folder for your experiment and images called Instant Imaging. After the imaging process, you can use Rock Maker to move the experiment file to a more appropriate location in the Explorer tree.

**Prerequisites**

- The plate is stationed on Rock Imager.

**To image with Instant Imaging:**

1. Start an Instant Imaging session: Go to the **Imager** menu and select **Instant Imaging**.

2. Check the imaging settings you want to use during the imaging process on the **Select Imaging Settings** dialog box.

3. Click **OK** to confirm. The window to define the experiment for the plate on the slot 1 opens.

4. Select your plate from the **Plate Type** list.

5. Name your experiment. The **Project Name** and **Folder** fields are automatically assigned.

6. Set the **Setup Temperature** and **Incubation Temperature**.

7. Tell Rock Imager which wells you want to image.

   - If you want to image all drop positions at each well, check the box next to **Full Plate**.

   - If you only want to select a certain drop location at each well, select the drop location you used (i.e. 1, 2) from the **Drop** list.

   - To image drops at specific wells, click the box at that location to check it.

   - Click **Select All** to image drops at all of the plate's wells. After clicking **Select All,** you can uncheck certain wells if you don't want to image them.

8. Click **OK** to complete **Instant Imaging** setup for the plate at slot 1. A window opens allowing you to select Instant Imaging options for a plate at slot 2.

   - **OPTIONAL**: Repeat steps 4-7 if you wish to image a second plate, or click **Cancel** if you do not have a second plate to image.

## Imaging a Plate with FRAP Benchtop

**Prerequisites**

- You have created an experiment in Rock Maker. (If you are attempting to image a plate without a barcode, please go to Instant Imaging).

- Your plate has a barcode label affixed to the short side of the plate opposite the A1 position.

**To image a plate:**

1. Open the FRAP door and place the plate(s) in the imager hand with the barcode(s) facing towards you.

2. Close the FRAP door.

3. Tell Rock Imager to scan the barcode.

   - Using the Rock Imager software, go to the **Imager** menu and select **Scan Plate(s)**.

4. Choose from the following options:

   - To image an entire plate with the default imaging setting, go to the **Imager** menu, point to **Image**, and select **Plate(s)**. Alternatively, go the **Scheduling** tab, right-click on plate(s) and select **Image Plate**.

- To image an entire plate with the imaging setting(s) of choice, go to the **Imager** menu, point to **Image**, and select **Plate(s) Using**. Rock Imager will display a dialog box that asks you to select available imaging settings (i.e. visible, High Throughput (HT) FRAP, and Full Recovery FRAP).

- To inspect your plates well-by-well or drop-by-drop without taking images, go to the **Live Image** tab and use the manual navigation controls.

- To image a specific drop with the default imaging settings, go to the **Imager** menu, point to **Image**, and select **Drop**.

- To image a specific drop with your choice of imaging settings, go to the **Imager** menu, point to **Image**, and select **Drop Using**.

- If you have tagged certain drops as interesting in Rock Maker and wish to image only those drops, go to the **Imager** menu, point to **Image**, and select **Interesting Drops** to use the default imaging setting or point to **Image**, and select **Interesting Drops Using...** to use a custom imaging setting[1].

- If you want to run Full Recovery FRAP imaging on the drop in view, go to the **Imager** menu and select **Full Recovery FRAP Current Position** to use the default settings or **Full Recovery FRAP Current Position Using** to select new settings.

5. When your plates have been imaged at least once, you can view the images in Rock Maker.

6. When you are ready to remove the plate(s) from FRAP:

- Go to the **Imager** menu and select **Load/Unload Plate**.

- Open the FRAP door.

- Remove the plate(s) from the imager hand.

---

[1]Requires Rock Maker 2.8

# Imaging a Plate with SONICC Benchtop

This topic explains how to image a plate from start to finish using the SONICC Benchtop version of Rock Imager. If you aren't using Rock Maker to track conditions, or you want to image a plate that does not have a barcode, please see Instant Imaging.

**Prerequisites**

- You have created an experiment in Rock Maker.
- Your plate has a barcode label affixed to the short side of the plate opposite the A1 position.

**Imaging a Plate**

1. Tell SONICC you want to load a plate. Go to the **Imager** menu and select **Load/Unload Plate**.

2. Place the plate on the imager hand. If you're using a barcode, the barcode must face towards you and should be affixed opposite the A1 position.

3. Choose from the following options:

    - To image an entire plate with the default imaging setting, on the **Imager** menu, point to **Image**, and click **Plate(s)**, or go the **Scheduling** tab, right-click on a plate and select **Image Plate**.

    - To image an entire plate with the imaging setting(s) of choice, on the **Imager** menu, point to **Image**, and click **Plate(s) Using**. Rock Imager will display a dialog box that asks you to select available imaging settings.

    - To inspect your plates well-by-well or drop-by-drop without taking images, go to the **Live Image** tab and use the manual navigation controls.

    - To image a specific drop with the default imaging settings, on the **Imager** menu, point to **Image**, and click **Drop**.

    - To image a specific drop with your choice of imaging settings, on the **Imager** menu, point to **Image**, and click **Drop Using**.

    - If you have tagged certain drops as interesting in Rock Maker and wish to image only those drops, on the **Imager** menu, point to **Image**, and click **Interesting Drop(s)** to use the default imaging setting, or point to **Image**, and click **Interesting Drop(s) Using** to use a custom imaging setting[1].

--------------------
[1] Requires Rock Maker2.8

## Imaging a Plate with RI 2

This topic explains how to image a plate with Rock Imager 2. You have two options: If you are using barcode labels on your plates, then follow the instructions in this section. If you want to image a plate that does not have a barcode label, please see Instant Imaging.

**Prerequisites**

- You have created an experiment in Rock Maker.

- Your plate has a barcode label affixed to the short side of the plate opposite the A1 position.

**Imaging a Plate with RI 2:**

1. Place the plate in any available slot with the label facing towards you.

2. Tell Rock Imager to scan the barcode: Go to the **Imager** menu and select **Scan Plate(s).**

3. Choose from the following options:

    - To image an entire plate with the default imaging settings, on the **Imager** menu, point to **Image**, and click **Plate(s)**.

    - To image an entire plate with the imaging setting(s) of choice, on the **Imager** menu, point to **Image**, and click **Plate(s) Using**. Rock Imager will display a dialog box that asks you to select available imaging settings.

    - To inspect your plates well-by-well or drop-by-drop without taking images, go to the the **Live Image tab** and use the manual navigation controls.

    - To image a specific drop with the default imaging settings, on the **Imager** menu, point to **Image**, and select **Drop.**

    - To image a specific drop with your choice of imaging settings, on the **Imager** menu, point to **Image**, and click **Drop Using**.

    - If you have tagged certain drops as interesting in Rock Maker, you can opt to image only those drops by going to the **Imager** menu, pointing to **Image**, and clicking **Interesting Drop(s)** to use the default imaging setting, or pointing to **Image**, and clicking **Interesting Drop(s) Using** to use a custom imaging setting[1].

4. When your plates have been imaged at least once, you can view the images in Rock Maker.

_____

[1] Requires Rock Maker 2.8.

---

## Viewing a Plate's Automatic Imaging

A plate's automatic imaging schedule and information can be seen at any time from the Imager and Scheduling tabs.
*Note: These instructions apply to RI 2, and SONICC and FRAP Benchtop. If you are using a different Rock Imager model, please refer to the instructions in Chapter 7.*

**Prerequisites**

- You have loaded and scanned the plate.

**To view a plate's automatic imaging schedule:**

- To view a plate's imaging schedule, go to the **Imager** tab and click **More** on **Plate Information**. Alternatively, right-click a plate on the **Scheduling** tab and select **Plate Information**.

---

## Interpreting FRAP Offset Information

During High-Throughput FRAP imaging, pre-bleach and post-bleach images are taken for each well, and then the stage positions the A1 drop back under the camera for end-state imaging. As FRAP results are calculated by analyzing the intensity change of the bleached area, and bleach spots are ~15 microns wide, an image alignment algorithm is used to account for the fact that the stage position is not repeatable from pre-bleach to end-state positioning on the same drop within 10 microns. The software algorithm repositions the end-state image to correctly overlay with the pre-bleach image. Expected values are approximately 10-30 µm. Image offsets between the pre-bleach and post-bleach images are also calculated. However, very minimal changes are expected here as the stage does not reposition in between the acquisition of these images.

Starting with Rock Imager 2.16, these offset numbers are available on the Imaging Settings panel in Rock Maker. These values are displayed for troubleshooting purposes.

**Viewing FRAP offset values:**

1. Using Rock Maker, locate the FRAP experiment in the **Explorer** and double-click it to open it.

2. Click the **Canvas** tab.

3. Double-click one of the drops. The drop image will appear in the **Drop** tab.

4. In the **Experiment** contextual tab set, click the **View** tab, and then click the **Imaging Settings** button in the **Info** group.

5. The **Imaging Settings** panel will appear next to the **Drop** tab. Look for "End-State image offsets" and "Post-Bleach image offsets". Offset values should range between 5-10 μm.
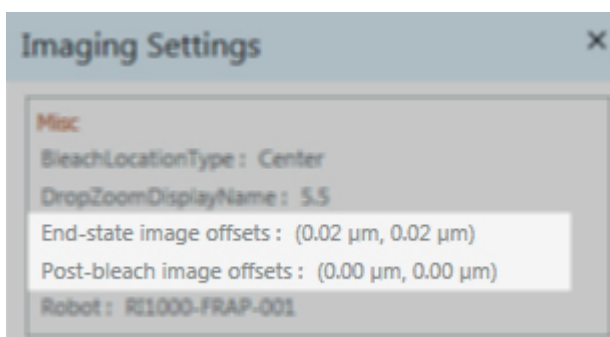


**Figure 8-5.** FRAP offset values in Rock Maker.

If the offset value differences are greater than ~30 μm, it may point to a problem with the stage or the image alignment algorithm. Email support@formulatrix.com for assistance.

## Interpreting SONICC Results

In general, the SHG imaging channel will provide the ability to quickly determine which wells contain protein crystals. However, because all samples are different, it is possible to have higher backgrounds than expected and signal from dust or salts resulting in false positives. The use of UV-TPEF provides the ability to rule out these false positives and to definitively determine whether or not regions of interest are protein crystals.

Tops of plates and sealing tape may generate significant amounts of SHG and fluorescence. If the laser is focused on these regions, then one may expect strong background interference from these materials. However, the depth of focus of the beam is small enough that when the laser is focused in the drop, there should be no out of plane contributions from the plates and tape.

Some salts that produce non-centrosymmetric crystals (either chiral or achiral molecules) will produce SHG making it difficult to distinguish between salt and protein crystals. The UV-TPEF channel is used to discern whether the crystal is of proteinaceous material. Those crystals that contain tryptophan will fluoresce when imaged with the 532 nm beam.

If both the SHG and UV-TPEF channels show similar contrast then you can be certain that your sample is a protein crystal (unless you have fluorescent small molecules present). Note that all protein material, whether crystalline or not, will show up on the UV-TPEF channel, so it is important to compare to the SHG channel to determine if it is crystalline.

The use of the UV-TPEF channel provides a clear assessment of each drop which can be seen in the figure on the next page. In both rows (a) and (b) contrast is observed in both the UV-TPEF and SHG channel indicating protein crystals. The well imaged in row (c) results in fluorescence but no SHG, indicating precipitated/aggregated protein. Salt crystals can also produce SHG as shown in row (d) but result in no fluorescence making it easy to distinguish between salt and protein crystals.
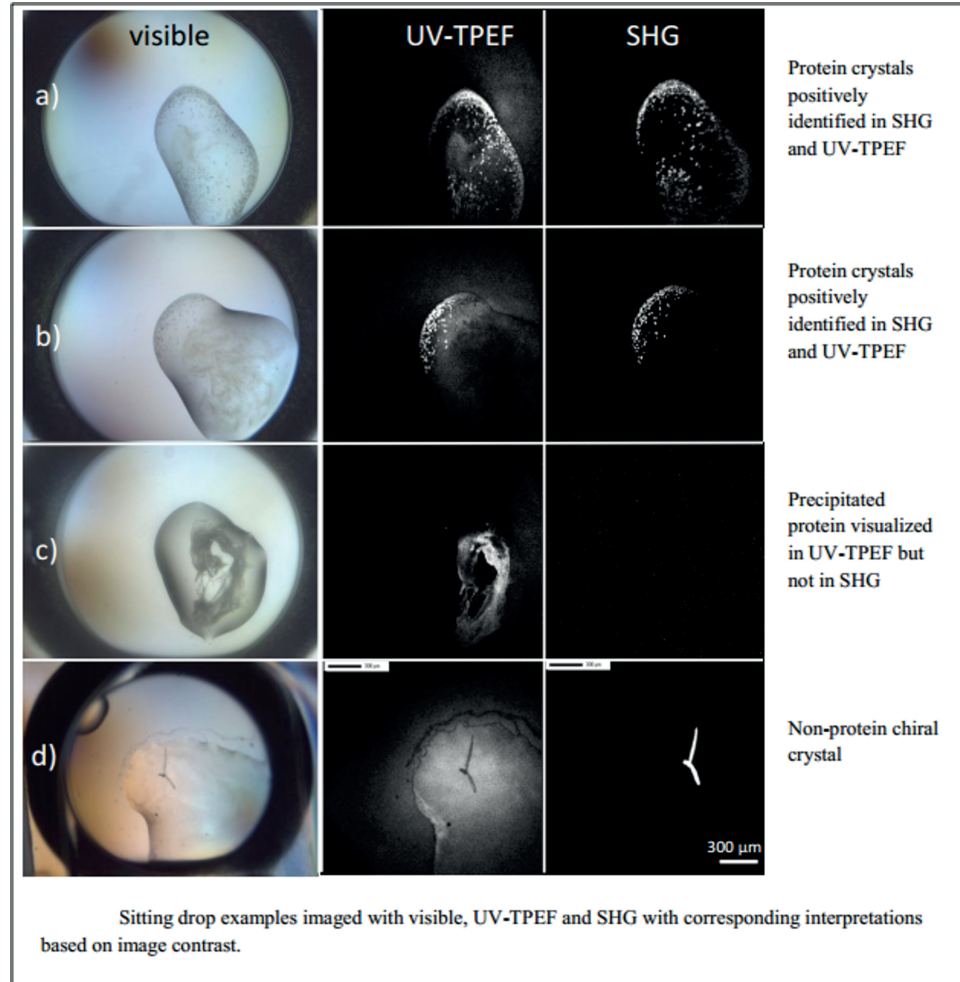
Sitting drop examples imaged with visible, UV-TPEF and SHG with corresponding interpretations based on image contrast.

**Figure 8-4.** SONICC Results.

## SONICC Reference Plate Image Analysis

In order to monitor and proactively care for the integrity of the laser, SONICC includes a SONICC reference plate to act as a control so you can be sure the laser is working at expected standards. The reference plate is made up of a 100 μm grid pattern overlayed on top of urea and tryptophan crystals, which give off ample SHG and UV-TPEF signal. The plate is imaged at set intervals in UV-TPEF, and average intensity is recorded and compared to initial results. If Rock Imager calculates a decrease in intensity of 30% or more, an email is automatically sent to Formulatrix support. A support engineer will then analyze your system to determine the cause of the degradation, fix any problems and/or replace the SONICC laser if necessary.

- RI-1000 Dual-Imager with SONICC Reference Plate Image Analysis -- Support technicians will store the plate inside the imager and execute the first Reference Plate Image Analysis session to create the benchmark data point. Rock Imager will then automatically image the reference plate daily with the same imaging settings.

- SONICC Benchtop Reference Plate Image Analysis -- As SONICC Benchtop lacks plate storage, image analysis is performed by support engineers at initial installation (beginning January 2016) to set the benchmark data point and at all subsequent support visits. However, bi-weekly tests should be performed manually by the lab manager or other designated personnel to properly monitor the integrity of the laser. The software will prompt you to run image analysis every two weeks; however, you can choose to delay the process or run it more frequently if desired.

**Initiating a Manual Reference Plate Image Analysis Session**

> **IMPORTANT**: SONICC Benchtops installed prior to January 2016 must have initial benchmark imaging performed with Formulatrix support technicians before initiating subsequent manual image analysis sessions.

1. SONICC Benchtop users must place the plate inside the imager before following these steps:

2. Go to the **File** menu, point to **Reports**, and then select **Reference Plate Image Analysis**.

3. Click the **Image and Analyze Reference Plate Now** button.

**Viewing/Exporting Analysis Results**

You can view reference plate analysis results at any time.

- Go to the **File** menu, point to **Reports,** and then select **Reference Plate Image Analysis**. Results are displayed in a table.

- If desired, you can export the data for use in programs such as Microsoft Excel by clicking the **Export Image Analysis Data** button.

# Configuring FRAP Imaging Settings
*Chapter 10*

FRAP imaging settings can be configured directly in Rock Imager, but be aware that these settings are overridden by Rock Maker imaging settings. It's a best practice to use the Rock Maker imaging setting setup in order to change the FRAP imaging settings, and to only adjust the settings discussed in this section under the guidance of Formulatrix support.

To access FRAP configuration settings, go to **File** and click **FRAP Configurations**. You may or may not be able to access the Servicing menu, as this menu is usually hidden in order to preserve the default FRAP settings.

## Bleaching Options

You will be presented with the FRAP Configurations dialog box that has three tabs: Bleaching Options, Imaging Options, and General.

On the **Bleaching** tab, you set options related to the laser, which is what bleaches the drop during FRAP imaging.

**Bleach Options**

The Bleach Options area provides two major settings, **Pulse Laser for** and **Drop area to include in Random Bleach Position**.

- **Pulse Laser for**. This field allows you to specify the amount of time the laser is focused on the drop to bleach the fluorophores.

- **Drop area to include in Random Bleach Position.** A bleach position is randomly chosen in the specified area. One or two different bleaching spots can be chosen; near the center of the drop or near the edge of the drop. Concentric rings define the boundary of the area in which a spot is chosen. If 25% area for Edge is specified, then a bleach spot will be performed on the outer 25% area of the drop, whereas the area for the center is defined from the center out.

**Full Recovery FRAP Post-Bleach Options**

In the Full Recovery FRAP Post-bleach Options section,  you modify settings related to Full Recovery FRAP imaging. Full Recovery should be used when you have identified interesting drops, as imaging takes longer since many more images are captured.

Generally, it's best to run High Throughput (HT) FRAP imaging first, and execute HT FRAP imaging once you identify interesting drops.

- **Acquire images at fastest rate for.** This setting defines how long, in seconds, the imager will take rapid pictures of your drop after laser bleaching is complete.

- **Interval.** This setting allows you to specify the number of seconds that will be used when taking additional images after the initial imaging process is complete.

- **Post-bleach imaging time.** This field is informational only, it is not configurable. It displays the number of seconds post-bleach imaging will take given the settings you configured on this tab.


**High Throughput FRAP Post-Bleach Options**

The High Throughput (HT) FRAP Post-Bleach Options area has only one setting, which is **Time to wait before taking end-state images**. The imager will pause for the specified number of seconds before beginning to capture images.


## Imaging Options

The FRAP Imaging tab offers the following settings:


- **Imaging Settings**. An imaging setting is a collection of settings that tells Rock Imager how an experiment plate should be imaged.  Imaging settings are configured in Rock Maker. The name of the specific imaging setting that has been set in Rock Maker for a Single Drop Full Recovery imaging session appears in the Single Drop Full Recovery box.

  If you change options here, you are changing the default settings. It's a best practice to train FRAP users to set up their desired imaging settings in Rock Maker, as you can have unlimited "Imaging Setting" profiles but only one default.

  Rock Imager will use the default FRAP settings, which are defined in Rock Imager, when no changes are made to the default Imaging Setting in Rock Maker. Once changes are made, Rock Imager ignores the default settings here.

- **Exposure Settings.** The exposure settings affects an images brightness. You can adjust fluoro light, exposure, gamma and gain settings. See Appendix B for information about how to use these fields.

- **Bright Field Focusing.** The Bright Field Focusing area is where you adjust bright field, gamma, exposure, gain, and condenser settings. See Appendix B for information about how to use these fields.

# General Options

General FRAP options include settings related to the curve generated based on the FRAP data.

- **Single Component Fitting.** Fits the pixel intensity recovery data with one Bessel function. The result of this numerical fitting is a single diffusion rate. This method is accurate enough for most applications, if protein molecules are labeled with dye.

- **Double Component Fitting.** Fits the pixel intensity recovery data with two Bessel functions. The result of this numerical fit is two distinct diffusion rates. This method is useful if the protein is not purified well. Some small molecule lipids are labeled with dye, which generate the two distinct diffusion rates: diffusion rate of lipid and diffusion rate of protein.

# Manual Inspections

*Chapter 11*

You can use Rock Imager's powerful optics to explore the contents of your plates at any time by switching from Automatic  Mode to Manual Mode and using the manual imaging controls. In this section, we explain how to manually image a plate and how to use the associated optics and camera controls.

## Manually Inspecting a Plate

**Prerequisites**

- You have loaded the plate to Rock Imager, scanned the barcode, and moved the plate to the desired imager (e.g. Visible, UV, FRAP, SONICC).

**To manually inspect a plate:**

1. Before you can use any of the manual imaging controls, you have to put Rock Imager into Manual Mode. To do so, go to the **Scheduling** menu and select **Manual Mode**.

2. Rock Imager asks you if it should automatically revert to scheduled imaging, or if you want to stay in manual mode indefinitely.

    - If you opt to stay in manual mode indefinitely, you will need to go to the **Scheduling** menu again later and select **Automatic Mode** in order for Rock Imager to continue with the automatic imaging schedule.

    - If you select **Automatically**, you'll need to enter the number of minutes in the appropriate field.

3. Click **OK**.

4. If you're not already on the Live Image tab, go to the **Imager** tab and click the **Live Image** sub-tab to open it.

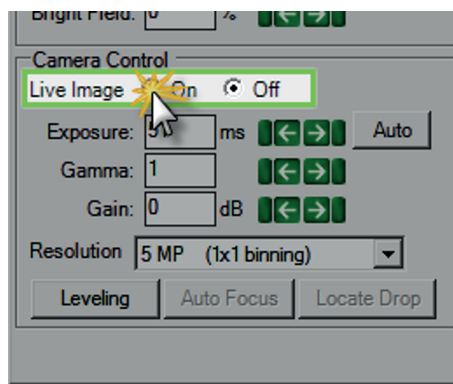5. Under **Camera Control**, set **Live Image** to **On** by clicking the radio button.



**Figure 10-1.** Camera Control

6. Use the **Well Controls** to explore the contents of your plate.

7. Use the **Optics Controls** and **Camera Controls** to fine-tune the live image.

## Manual Imaging Controls

**Imager Control**

The Imager Control panel is part of your software build when you have more than one imaging method inside your system. Use it to switch between available imaging methods. You might find it useful to locate a drop using Visible light and then imaging it with UV, FRAP, or SONICC, for example.

The Imager Control panel, when displayed, is found on the Live Image sub-tab of the Imager tab at the top of the right-hand side of your screen.
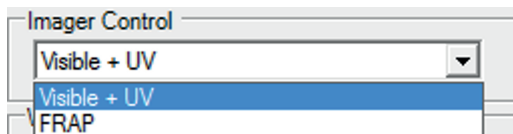


**Figure 10-2.** Imager Control panel.

**Well Control**

Well Controls are found on the **Live Image sub-tab** of the **Imager tab**, at the top of the right-hand part of your screen. Use the Well Controls to navigate around your plate to the drop of interest.



**Figure 10-3.** Well Control panel.

- To move from well to well, click the radio button next to Well and use the green control pad to navigate.

- To move around within a drop, select the drop number next to the Drop field and then click a radio button next to either Fast, Medium, or Slow and use the green control pad to navigate.
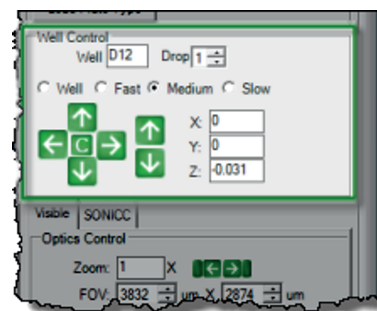
You can also navigate around your plate using the number pad on your keyboard. 4 and 6 move you along the X axis, 8 and 2 move you along the Y axis, and 9 and 3 move you along the Z axis.

The default speed for keyboard controls is slow. Hold down the `CTRL` key on your keyboard to change the pan speed to medium. Hold down the `ALT` key to change the pan speed to fast.

**Camera Controls**

Camera Controls are used during manual imaging sessions to adjust the way your images appear on the screen. Adjusting these controls does not affect the way your images are captured during automatic scheduled imaging sessions, as those parameters are defined by the imaging setting(s) selected in Rock Maker.

The Camera Control panel is found on the **Live Image** sub-tab of the **Imager** tab.

The contents of the Camera Control panel vary depending on the imaging method you are using. There are three possible Camera Control panels, as shown in the figure on the next page. If you have the SONICC Rock Imager, for example, your Camera Control area will show one additional field called Laser, and the Auto Focus and Locate Drop buttons are hidden.

There are three variations of camera controls (refer to Figure 10-5). See Appendix A for information on how to use Camera Controls.

**Optics Controls**

The Optics Control panel contains controls that allow you to manipulate the microscope's optics while manually inspecting a plate. The optics controls appear on the left-hand of the **Live Image** sub-tab of the **Imager** tab, and vary based on the imaging methods you purchased.

There are six possible optics control panels, as shown on the opposite page. See Appendix B to learn how to use the controls.

**Leveling**

One of the buttons on the Camera Control panel is the **Leveling** button. Clicking it opens the **Leveling Histogram**, which you use to adjust the brightness and contrast of an image while you are viewing a drop in either Manual Mode or Automatic Mode.

The changes you make with the leveling histogram are display changes; they are not saved to the original image. However, you can adjust image brightness and contrast with the leveling histogram in Rock Maker after your images have been processed by Rock Imager Processor (RIP).
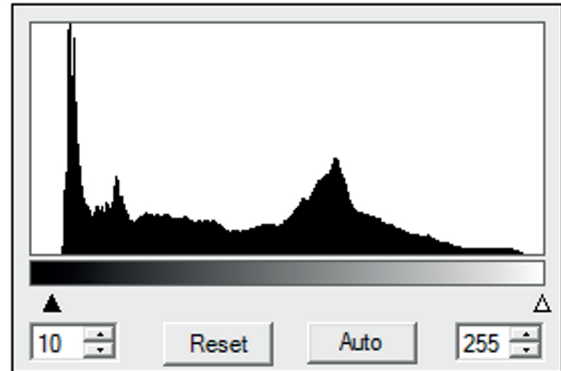


**Figure 10-4.** Leveling Histogram.

The histogram works through two parameters, the Lower Limit and the Upper Limit, which are indicated by triangles below the histogram. You can drag each of these triangles to the left or right to adjust the starting and ending histogram values.

**Setting the Leveling Histogram Values**

You can either adjust leveling for images on an individual basis, or adjust leveling for one image, right-click and select **Save Current Threshold for Auto-Leveling**. Once you set the initial auto-leveling values, Rock Imager attempts to emulate the resulting brightness and contrast for every image in that inspection when you click the **Auto** button.
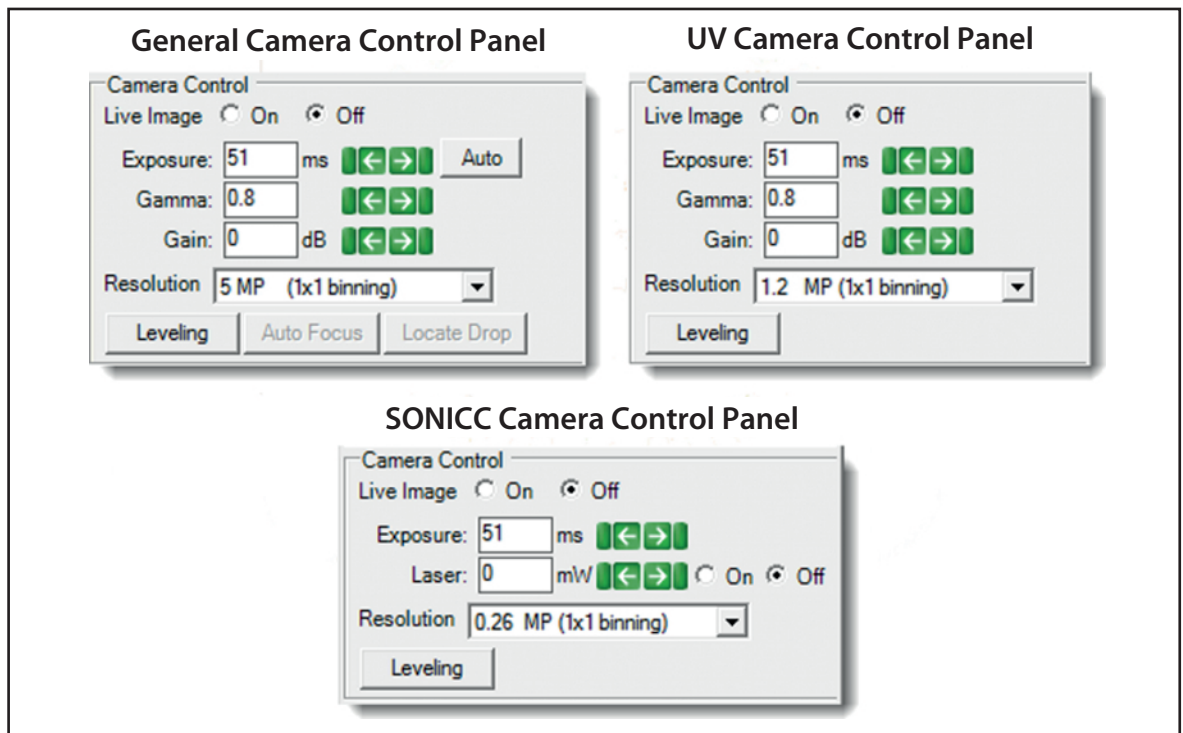
## General Camera Control Panel

Camera Control
Live Image ○ On ⦿ Off
Exposure: 51 ms [|←][→|] Auto
Gamma: 0.8 [|←][→|]
Gain: 0 dB [|←][→|]
Resolution: 5 MP (1x1 binning) ▼
Leveling | Auto Focus | Locate Drop

## UV Camera Control Panel

Camera Control
Live Image ○ On ⦿ Off
Exposure: 51 ms [|←][→|]
Gamma: 0.8 [|←][→|]
Gain: 0 dB [|←][→|]
Resolution: 1.2 MP (1x1 binning) ▼
Leveling

## SONICC Camera Control Panel

Camera Control
Live Image ○ On ⦿ Off
Exposure: 51 ms [|←][→|]
Laser: 0 mW [|←][→|] ○ On ⦿ Off
Resolution: 0.26 MP (1x1 binning) ▼
Leveling

**Figure 10-5.** Camera Controls.

## Visible Light Optics Controls

Optics Control
Zoom: 1 X [|←][→|]
FOV: 0 um X 0 um
Condenser: 0 % [|←][→|]
Polarizer: 0 ° [|←][→|]
Bright Field: 0 % [|←][→|]
Illumination: ☐ BrightField ▼

## FRAP Optics Controls

Optics Control
Zoom: 1.1 ▼ X [|←][→|]
FOV: 828 um X 621 um
Condenser: 0 % [|←][→|]
Polarizer: 0 ° [|←][→|]
Bright Field: 0 % [|←][→|]
Fluoro Light: 0 % [|←][→|]
Laser: ○ On ⦿ Off
Illumination: ☐ BrightField ▼

## UV Optics Controls

Optics Control
Zoom: 3.6 ▼ X [|←][→|]
FOV: 1022 um X 767 um
Illumination: ○ On ⦿ Off

## SONICC Optics Controls

Optics Control
Objective: 10x 0.15NA ▼
Zoom: 1 X [|←][→|]
FOV: 2700 um X 2025 um
Sensor: SHG ▼

## SLP Visible Light Optics Controls

Optics Control
Condenser: 0 % [|←][→|]
Bright Field: 0 % [|←][→|]

## SLP UV Optics Controls
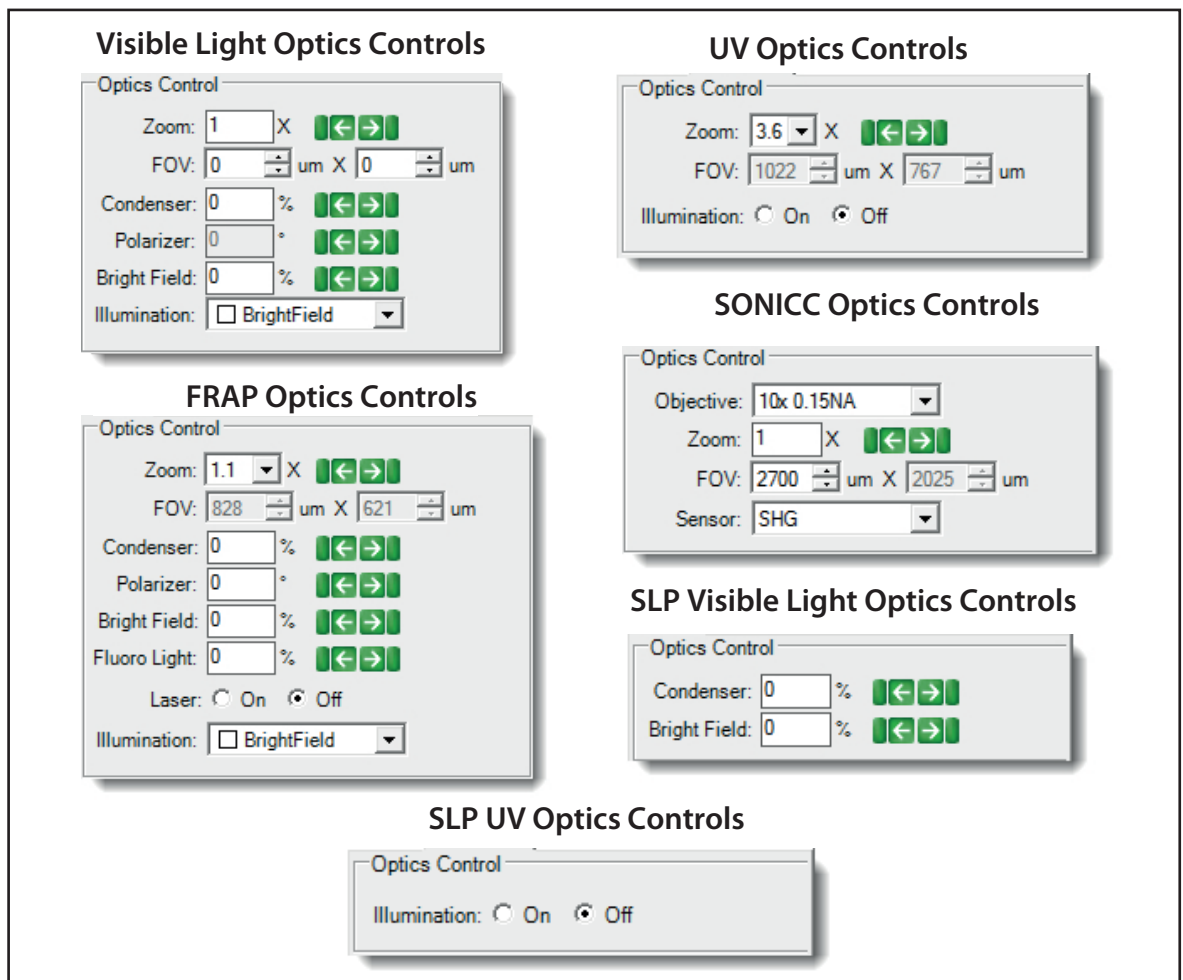
Optics Control
Illumination: ○ On ⦿ Off

**Figure 10-6.** Optics Controls.

**To adjust limits:**

- Click and drag the triangles on the Leveling Histogram control to the right or left to increase or decrease their settings.

    **OR**

- Type a new value into the related field and press **ENTER** on your keyboard.

    **OR**

- Click the up and down arrows to increase or decrease the values.

**Tips**

- Click the **Reset** button to reset any changes made to the image.

- To reposition the Leveling Histogram control, hold down the **SHIFT** key on your keyboard and click-and-drag the image to move it.

- To reset the position of the Leveling Histogram, right-click on the histogram and select **Dock**.

- As a starting point, try dragging the lower level up to point where your histogram begins and the upper level down to the point where it ends.

## About Auto Focus

When you click the **Auto Focus** button, you instruct Rock Imager to execute the Auto Focus algorithm. Auto Focus locates objects in a drop along the Z-axis, which is especially important when imaging hanging drops on greased cover slides, or when zooming in on a region of interest.

**How Does Auto Focus Work?**

The imager captures a stack of images at 30 frames per second at a low resolution and computes a focus curve. The imager then determines the most in-focus image and captures that image at the normal resolution.

You can adjust the focus levels for each imager in the Focus Level Selection area of the Plate Type Editor.

There are three Capture Range options:

- Auto Focus with Limits

- Fixed Range (DoF Limited), and

- Fixed Range (Interpolated)

**Auto Focus with Limits**

The first four fields (Max. focus levels above, Focus threshold above, Max. focus level below, Focus threshold below) determine the "stack" of images to be taken around what Rock Imager determines to be the best focus point. These settings are configured on a per-plate basis by our technicians when Rock Imager is installed, but you can adjust them.

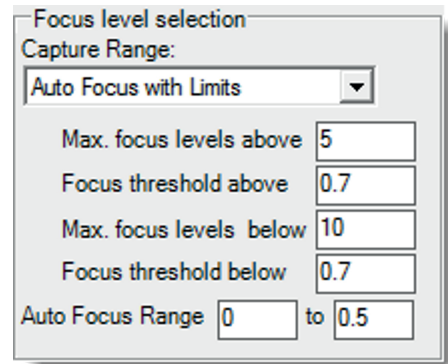Auto Focus with Limits factory settings for a 3 Drop Intelliplate are as seen at right.



**Figure 10-7.** Focus Level Selection panel.

**Fixed Range (DoF Limited)**

If you decide to use the Fixed Range (DoF, or Depth of Field Limited) capture range, you will configure the Start Z and Stop Z positions and the software will determine the appropriate number of focus levels.

**Fixed Range (Interpolated)**

If you decide to use the Fixed Range (Interpolated) capture range, you can configure the Start Z and Stop Z positions and the maximum number of focus levels that the imager should take.
**Note:** Additional focus levels mean additional imaging time, and vice versa.

# About Drop Location

When Rock Imager executes drop location on a drop, the drop area is imaged with a large field of view, and the lighting is set in order to create shadowing within the drop. Rock Imager then analyzes the image to locate the contrast between the shadow in the drop and the rest of the image, and zooms in on the shadowed area. The imager then uses normal imaging setting settings to photograph the drop.

The Drop Location algorithm can be executed from the **Camera Control** panel during a manual plate inspection. It's also executed on a per-plate or per-drop basis, for every imaging session or only the first time a plate is imaged, during automatic imaging sessions. Automatic imaging drop location settings are controlled from the **Plate Type Editor** – see the next chapter for more information.

You can view the results of the Drop Location algorithm on the **Drop Location** sub-tab of the **Imager** tab. If Rock Imager has located a drop, a red box will appear around the drop. (The red plus symbol indicated the center of the drop; the green plus indicates the center of the image.)

# Improving Images
## *Chapter 12*

Formulatrix technicians tune the settings for each type of plate you plan to use with your Rock Imager during initial installation. However, if you start using new plate types, or you notice your imager isn't capturing drop images as well as it once was, you may want to fine-tune these settings to improve drop location accuracy using the Plate Type Editor.

In this section, you will learn about:

- Using the Plate Type Editor
- Applying Image Masks
- Improving Drop Location
- Clearing Drop Locations

## Using the Plate Type Editor

The Plate Type Editor contains settings for all of your plate types. While most of the fields on the editor are configured during installation, we encourage you to adjust the settings for the best performance from your imager.
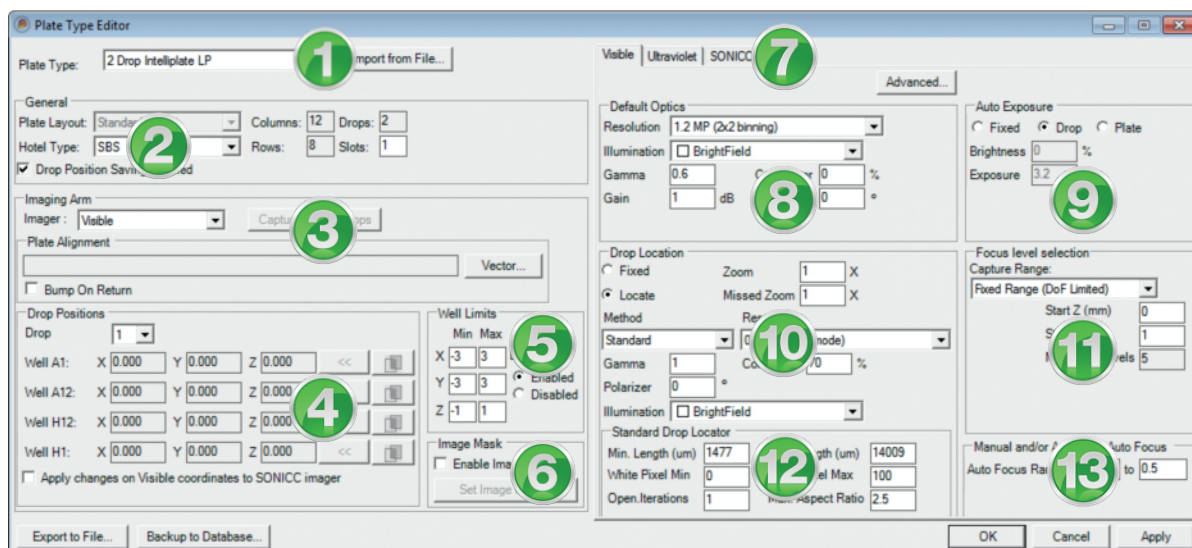


**Figure 11-1.** The Plate Type Editor.

**1** Plate Type

Select the plate you're interested in editing from the **Plate Type** list. You can add new plate types to the Plate Type list from the Rock Maker software (see **Adding a Container (Plate Type)** in the Rock Maker help files). All other fields on the Plate Type Editor are dependent upon the Plate Type selected. Default definitions for each plate type are available by importing a plate definition .xml file (obtainable by support@formulatrix.com), or you can import a previously saved definition file. To import a plate definition file, click the **Import from File** button, select the desired plate definition (the plate definition should match the actual plate type you would like to use), and click **Open**. The Plate Type Editor reads the file and applies the information to each field on the Plate Type Editor for the plate type. From there, you can edit the fields on the Plate Type Editor if necessary. X,Y,Z coordinates for the four corner well locations can only be updated if that specific plate type is registered with a barcode and on the imager hand.

**2** General

The General area of the Plate Type Editor includes basic information about the specifications of the plate, including the number of Columns, Rows, Drops per well and the number of hotel slots required for the plate.

The Drop Position Saving Enabled checkbox determines whether or not the Drop Location Algorithm will run each time this plate type is imaged, or if Rock Imager will save the settings to memory. Imaging time is faster when Drop Position Saving Enabled is checked.

> If your drop no longer fits in the drop position Rock Imager determined because of drop growth, uncheck Drop Position Saving Enabled.

**3** Imaging Arm/Plate Alignment

The Imaging Arm area provides two options for the imager used in the system. Please note that this option is available only if you purchased a dual imager system. The Plate Alignment field contains a file that helps Rock Imager position the plate for imaging. If you have SONICC imager, you can use the Capture Tuned Drops button to copy drop positions with the visible imager to SONICC. Bump on Return allows you to enable or disable the plate alignment when returning plates from imager to storage or load port.

**4** Drop Positions

The coordinates in the Drop Positions field correlate to the corner drops on the plate. From the coordinates of these corner positions, Rock Imager uses linear interpolation to compute the locations of all drops on the plate. You can tune these positions for better images at any time.(see Improving Drop Location).

If you have a SONICC imager,  check the **Apply changes on Visible coordinates to SONICC imager** box to apply any drop location changes to both plate definitions (Rock Imager maintains separate plate definitions for dual imagers).

**5  Well Limits**

The height limits in millimeters (mm) imposed on the X, Y and Z axes when you are manually inspecting drops.

**6  Image Mask**

Image Masks are related to Auto Scoring, a feature of Rock Imager 2.9 (requires Rock Maker 2.7). Certain plate types fluoresce causing false positives. You can "mask" a plate to increase Auto Score accuracy, which will hide the edges of a plate and instruct Rock Image Processor to ignore florescence from those areas when computing a positive or negative score for the drop.

**7  Imager Selection Tabs**

The number and type of imagers available here depends on the imaging types you purchased. Visible is always present; optional tabs are Ultraviolet, FRAP and SONICC. The fields on the right of the Plate Type Editor depend on the imager tab selected.

**8  Default Optics**

The Default Optics field contains the settings for the optics components and resolutions used during drop imaging, including Gamma, Condenser and Polarizer settings.

**9  Auto Exposure**

Auto Exposure settings determine how exposure settings are determined by Rock Imager during plate imaging.

- Selecting **Fixed** instructs Rock Imager to use the Brightness and Exposure settings in the corresponding fields.

- Selecting **Drop** instructs Rock Imager to run the auto exposure algorithm and determine exposure for each drop prior to imaging each individual drop on a plate. This is the most common setting.

- Selecting **Plate** instructs Rock Imager to run the Auto Exposure algorithm on the first drop on the plate and use those exposure settings for every drop on the plate thereafter.

**10** **Drop Location**

Drop Location settings instruct Rock Imager how to execute the Drop Location algorithm.

- Selecting **Fixed** instructs Rock Imager to use the drop positions defined in the Drop Positions field with the defined zoom (see number 4). If the plate type you select has two drops per well, you can configure the zoom for both drops 1 and 2 through the **Advanced** option

- Selecting **Locate** instructs Rock Imager to use the Drop Location algorithm to locate drops and enables you to control the illumination pattern such as top-left, top-right, bottom-left, darkfield, and brightfield to be projected onto a drop through the **Illumination** list. If you select Locate, you must also select a method from the **Method** list. You can select Standard or Lipid Phase as a method.

**11** **Focus Level Selection**

Think of Focus Level Selection as a capture range. These settings determine how Rock Imager images a drop once it locates one. You can select **Auto-focus with Limits**, **Fixed Range (DoF Limited)**, or **Fixed Range (Interpolated)**.

- If you select **Auto Focus with Limits**, Rock Imager runs the Auto Focus algorithm on the drop to pinpoint the best focus level for the drop on the Z axis. Once the focus level is determined, Rock Imager captures images above and below this point, called "slices". The slices are analyzed and compiled by Rock Imager Processor into the EFI image, which is the default image for the drop in Rock Maker.

  The three settings below the Focus level selection determine how the slices are captured. The Start Z and Stop Z values determine the range along the Z axis where Rock Imager captures slices of the drop. The Max focus levels settings equates to how many slices Rock Imager captures before sending the images to Rock Imager Processor.

- If you select **Fixed Range (DoF Limited)**, Rock Imager will capture slices along the Z axis between the start and stop heights and also determines the best number of slices to take.

- If you select **Fixed Range (Interpolated)**, Rock Imager captures a series of slices along the Z axis between the start and stop values of the Auto Focus range heights. In this case, you set the number of slices Rock Imager takes in the Max focus levels field.

**12** **Standard Drop Locator**

Standard Drop Locator settings (available from the Visible tab) instruct Rock Imager how to locate drops before imaging. You can define Min Length (μm) , Max Length (μm), White Pixel Min, White Pixel Max, Open Iterations, and Max Aspect Ratio.

- **Min Length (μm).** Controls the minimum number of μm  in a blob for that blob to count as a drop.

- **Max Length (μm).** Controls the maximum number of μm in a blob for that blob to count as a drop.

- **White Pixel Min.** The minimum range of pixel intensities maps to white when converting a sobel image to a binary image.

- **White Pixel Max.** The maximum range of pixel intensities maps to white when converting a sobel image to a binary image.

- **Open Iterations.** Determines the number of iterations the system runs when opening the image in preparation for drop location.

- **Max Aspect Ratio.** The maximum ratio between the largest and the smallest diameter of a blob that represents the drop.

**(13)  Manual and/or Automatic Auto Focus**

The Manual and/or Automatic Auto Focus setting (available from the Visible tab) enables you to limit the movement of Auto Focus. By default, you can adjust value in 0 - 0.5.

## Applying Plate Masks

Plate Masks are designed to improve Auto Scoring results by "masking" out fluorescence that appears around the edges of certain plate types. Although it is called a plate mask, you will set the mask using a particular drop on the plate. Once you set the plate mask for a single drop, the mask will apply to all drop locations on the plate -- hence the name plate mask.
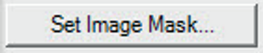
**Prerequisites**

- You are using a UV or UV-TPEF imaging method

- You are using a plate that autofloresces.

**To create a plate mask:**

1. Go to the **File** menu and select **Plate Type Editor** to open the Plate Type Editor.

2. Select the plate that you want to create an image mask for from the **Plate Type** list.

3. Check the box next to **Enable Image Mask**.
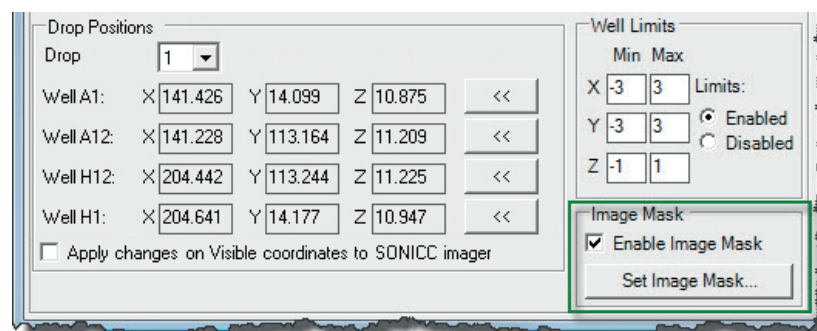
4. Click the [Set Image Mask...] button.

5. Select a shape from the **Shape** list. The shape should match the shape of your well. Options include circle, square, rectangle, rounded square, or triangle.

6. Set the size and position of the plate mask with your mouse. The irrelevant part of the plate should be outside the region you set.

7. Click **OK**.

> If the image in the Plate Image Mask window is not clear enough, click the Reload From Live Image button.

## Improving Drop Location

Although our technicians tune your plate type definitions during installation, if you add new plates types or want to tune the drop location for more precise images, you may want to improve the drop location algorithm.
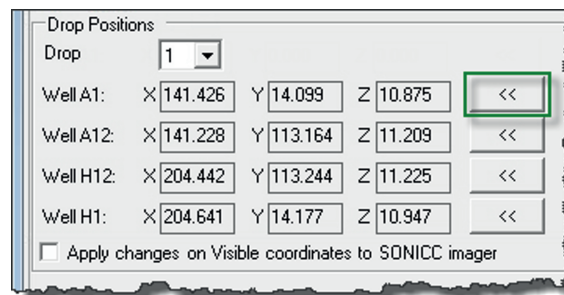


**Figure 11-2.** Rock Imager 2

**Prerequisites**

- You're comfortable using the Plate Type Editor.

- You know the name of the plate you want to improve the drop location algorithm on.

**To improve drop location:**

1. On the **File** menu, click **Plate Type Editor** to open the Plate Type Editor.

2. Select your plate type from [<<] the list.

3. If your plate has more than one drop location per well, select the number of the drop position you want to improve from the **Drop** list.

4.   Use the manual imaging controls to move the imager to the A1 drop location on the plate.

5.   Click the double-arrow button  to save the new drop position.

6.   Repeat steps 4 and 5 for the A12, H12, and H1 drop locations.

7.   Click **Apply**.

## Clearing Drop Locations

Occasionally you may need to clear or reset a drop location on a per-drop or per-plate basis.

*Note: Whether or not Rock Imager executes the drop location algorithm on a plate is determined by settings in the Plate Type Editor.*

**Prerequisites**

- Rock Imager has already identified drop locations on your plate.
- You want to remove or reset one or all drop locations.

**To clear all drop locations on a plate:**

1.   Locate the plate using any one of the **Plates** sub-tabs.

2.   Right click on the name of your plate and select **Clear Drop Location Positions**.

**To clear a single drop location:**

1.   Locate the plate using any one of the **Plates** sub-tabs.

2.   Right-click on the name of your plate, point to **Move Plate to**, and click **Imager**.

3.   Using the manual imaging controls on the **Live Image** sub-tab of the **Imager** tab, navigate to the drop.

4.   On the **Imager** menu, click **Clear Drop Position on Current Drop**.

# Rock Maker: The Crystallography Center
*Chapter 13*

Rock Maker is a complex, powerful laboratory information management system. Using Rock Maker, you can design screens and experiments; print barcode labels for plates and ingredient bottles; dispense plates (if Rock Maker is integrated with a liquid handling robot); view and score images; track conditions; optimize around conditions; and mine data using Search and Filter. Rock Maker is also where you can create custom imaging settings and create and edit imaging schedules.

To use Rock Maker experiments, you only need to know a few Rock Maker basics, listed below:

- How to create an experiment and define imaging settings in Rock Maker.

- How to print a barcode label for your plate in Rock Maker.

- How to view and score images in Rock Maker.

## Creating an Experiment in Rock Maker

To create an experiment in Rock Maker, you will need to create a protein formulation, define the volumes and locations on your plate, and add a screen. Rock Maker includes walk-through tutorials that assist you with these processes via a feature called *Rock Maker University* (RMU). RMU includes both tours and tutorials – a tour is indicated by a tour bus; tutorials are indicated by graduation caps. Tours are informative. Tutorials assist you with completing a task. Once you learn how to perform basic tasks, you can create experiments and proteins on your own. If you need to create both a protein formulation and a screen formulation, follow the instructions below. If you already have a protein formulation, skip to "Creating a Screen Experiment".

You will be asked to select an imaging schedule during the "Create a Screen Experiment" tutorial. An imaging schedule is only necessary if you want to image the plate over a series of time and plan on leaving it inside Rock Imager. However, you must select the imaging settings regardless. The imager will use the imaging settings you select for 0 days, 0 hours.

**Prerequisites**

- You are running Rock Maker version 3.0 or newer.

**Creating a protein formulation:**

1.  On the Rock Maker main window, click the **Rock Maker University** button ![icon].

2.  Point to the **Design** section.

3.  Select **Create Protein Formulation**.

4.  Follow the on-screen instructions. At the end of the protein formulation tutorial, click **GO!** to immediately begin creating a screen experiment.

**Creating a screen experiment:**

1.  On the Rock Maker main window, click the **Rock Maker University** button ![icon] .

2.  Point to the **Design** section.

3.  Select **Create Screen Experiment**.

4.  Follow the on-screen instructions.

# Printing a Barcode Label for a Plate

Once you have designed and edited your experiment, you must print a barcode label for the plate.

**Prerequisites**

- You are running Rock Maker version 3.0 or newer.

**To print a barcode label:**

1.  On the Rock Maker main window, locate the experiment you created in the **Explorer**.

2.  Click the small black triangle next to your experiment to display the experiment plate. The experiment plate is indicated by a gray plate image followed by a string of numbers, which is the numeric value of the barcode label you are about to print.

3.  Right-click on the plate and select **Print Barcode Label**.

4.  Affix the label to your experiment plate. It must be placed on the short side of the plate opposite the A1 position. Make sure the barcode label is not hanging over the edges of the plate; this may interfere with imager operation.

# Viewing and Scoring Images in Rock Maker

Once **Rock Imager** has taken images, you view them with **Rock Maker**. Although you should refer to the Rock Maker help files for more details, this topic provides a quick overview about how to access and view your images with the Rock Maker software.

If an experiment has images, the experiment will automatically open to Image View when you open the plate. However, you can access images view at any time through the following methods.

**Prerequisites**

- You are running Rock Maker version 3.0 or newer.

- You created an experiment in Rock Maker.

- You imaged the experiment's plate one or more times with Rock Imager.

**To view Images in Rock Maker:**

1. In the **Experiment** contextual tab set, click the **View** tab, and click the **Image View** button in the **Canvas Mode** group.
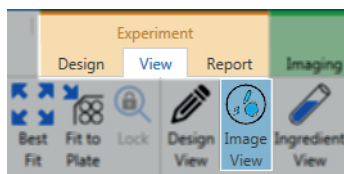


2. Click the **Image View** button on the **View** bar.



3. Click the **Navigator** button on the **View** bar to be able to look at image thumbnails and move around from one image to the next.



The Navigator contains all controls necessary to browse through plates, wells, drop, regions, imaging settings and imaging sessions. Two modes are available on the Navigator, *Thumbnail View* and *Composite View*. Thumbnail View is the first mode you see. Composite View is accessible by clicking the Composite View button at the bottom-left of the Navigator. To bring up the full-size image of any thumbnail you see, double-click the thumbnail.
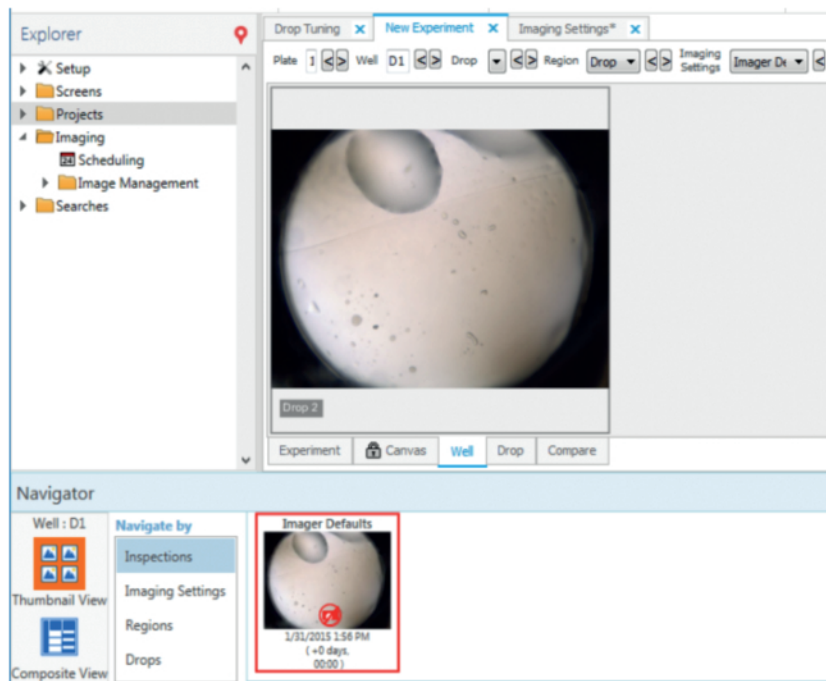
- To see each of the z-slices for a drop, go to the **Drop** tab and use the `F11` and `F12` keys on your keyboard.

- To compare an image with another image, press and hold the `CTRL` key on your keyboard, and click on the images you want to compare.



- To change the way you are navigating through images, use the **Navigate by** list.
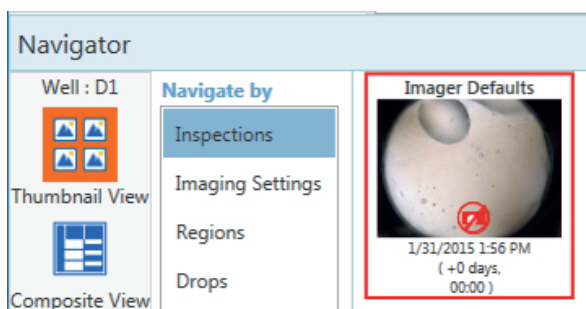
- If your plate has multiple drops per well, you must change the **Drop** list on the **Experiment Navigator**.
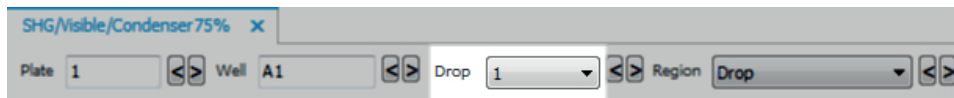


- To adjust the brightness and contrast for your images, click the **Tools** tab in the **Images** tab set and use the **Image Tuning** controls. Drag the red and green lines in between the histogram to the left-most part of your image's histogram and the right-most part for the best contrast and brightness.
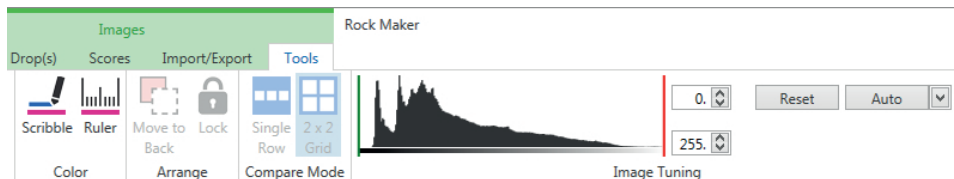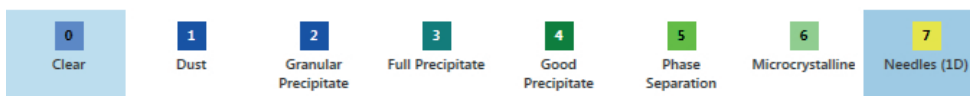


**To score images:**

1. While in Images view, select a drop by clicking it. If you want to assign the same score to multiple drops, select them all from the **Canvas** tab.

2. Press a number on your keyboard to assign a score. Rock Maker moves you to the next image.

   **OR**

   Back in the **Scores** tab, click the desired score button. Rock Maker moves you to the next image.

# FAQs
## *Chapter 14*

If your question does not appear below, please email support@formulatrix.com and we will respond within 24 hours, Monday through Friday.

**Q:  How do I change the imaging schedule?**

Since schedules are defined in Rock Maker, this task is accomplished using the Rock Maker software. Once in Rock Maker, click the Experiment tab. You can adjust the schedule in the Imaging Schedule and Imaging Settings area.

**Q:  I have a plate that I want to image right away. How do I do it?**

1.  Insert your plate in the load port.

2.  Scan the barcode by going to the **Load Port** menu and selecting **Scan Load Port**.

3.  Move the plate to the imager by going to the **Imager** menu, pointing **Image**, and clicking **Plate** to image the plate with the default imaging setting or pointing to **Image**, and clicking **Plate Using...** to image the plate with a new imaging setting.

**Q:  How do I image a drop I'm viewing in manual mode?**

On the **Imager** menu, point to **Image**, and click **Drop** if you want to image the drop with the default imaging setting, or point to **Image**, and click **Drop Using** if you want to use custom imaging settings.

*Q: How do I add a new plate type?*

Please contact us by sending an email to support@formulatrix.com that includes information about the plate type you wish to add. One of our support technicians will respond with a plate type definition file and explain how to add it to your plate library.

*Q: Can Rock Imager notify me when a plate has completed its automatic imaging schedule?*

Rock Imager can't, but Rock Maker can.

1. Using the **Rock Maker** software, go to the **Explorer** and open the **Setup** node and then click **Users**.

2. Add or change the email address and select **Immediate** or **Daily Digest** from the **Notification Type** list.

*Q: The drop locations Rock Imager found on my plate aren't accurate. How do I improve them?*

If you are using automatic drop location, see Clearing Drop Locations for information on how to clear the current drop locations.

If you are using fixed drop location:

1. On the **File** menu, click **Plate Type Editor**.

2. Select your plate type from the Plate Type list.

3. If your plate has more than one drop location per well, select the number of the drop position from the **Drop** list.

4. Use the manual imaging controls to move the imager to the A1 drop location.

5. Click the **<<** button (outlined in green below) to save the new drop position.
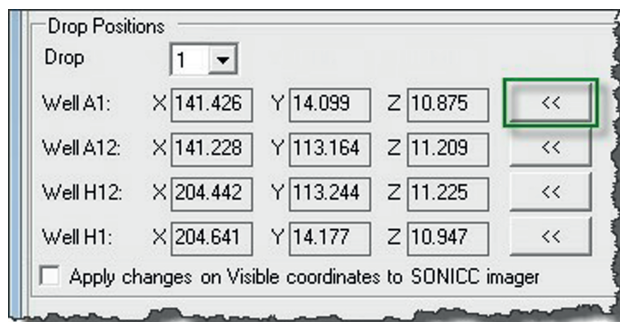


6. Repeat steps 4 and 5 for the A12, H12, and H1 drop positions.

7. Click **Apply**.

*Q: Why aren't my images showing up in Rock Maker?*

- Check to make sure that Rock Imager Processor (RIP) is running by looking at your status bar. If RIP is off, please start it.

  If RIP was installed on your local computer, there should be a shortcut on your desktop. You can also locate the Formulatrix folder and look for a file named RockImagerProcessor.

  If you are on a network, RIP may be installed on another computer. If you don't know which computer RIP is installed on, please contact your administrator or Formulatrix support.

- Try clicking the **Refresh Images** icon on the Rock Maker Toolbar.

- Check your network connections.

- If your images still aren't showing up, contact support by emailing support@formulatrix.com.

*Q: How do I save the image I'm seeing on the Live Image tab? CTRL+S isn't working.*

In Rock Imager, `CTRL+S` tells the imager to store the contents of the load port. To save the image you're viewing, go to the **File** menu and select **Save Image.**

*Q: Rock Imager can't find my plates in the load port. What do I do?*

First, check that the barcode label is facing away from you towards the imager on the short side of the plate opposite the A1 well position. Second, check that no part of the barcode label is stuck to the bottom of the plate or wrapped around a corner of the plate. Then, go to the **Load Port** menu and select **Scan Load Port**.

*Q: How do I move my plates from the load port to storage?*

Go to the **Load Port** menu and select **Store Contents**, or use the keyboard shortcut `CTRL+S`.

## UV FAQs

**Q:  What plates and cover material are compatible with UV?**

Plates vary, and can even vary within a brand. The most confident answer is that polyolefin works fantastically, and polystyrene can vary from not good to OK. For more information, please see the Formulatrix plate guide (http://www.formulatrix.com/plateguide/).

**Q:  What is the smallest protein crystal that the UV camera can pick up?**

This answer is dependent upon your plate. On a perfect plate, the answer is  <2 μm.

**Q:  Why do some proteins fluoresce more than others?**

Multiple causes. The signal is directly proportional to the concentration of tryptophan. If a protein does not have many tryptophan residues, then it would be expected to have less signal.

Additionally, plate media can dampen the signal, or introduce noise that when compensated for leaves the impression of less fluorescence, which is why plate masking is important for UV imaging. Also, the drop fluid itself could be absorbing inbound excitation UV or outbound emission UV.

**Q:  What concentration of tryptophan is needed to make the protein crystal fluoresce under UV?**

Technically, any tryptophan will fluoresce, at any concentration. As far as what concentration threshold is needed in order for the florescence to translate to the image captures -- the answer varies with exposure time, ambient light, gain setting, plate media interference, etc.

**Q:  Can we adjust the brightness of the UV LED?**

No. The LED is at it's max without burning out as it is. Too much LED will cook your protein samples.

**Q:  Do glass cover slides degrade the fluorescence?**

Some interfere, some don't. The best way to check is with a spectrophotometer.

**Q:  Can you get false negatives or false positives in terms of determining if a crystal is a protein or not?**

False Positives: Some other things fluoresce -- especially if they have aromatic rings in their chemical structure. This includes some detergents, some plastics (polystyrene), skin cells (trytophan, in that case), and even the artificial sweetener saccharin. Generic room dust contains an impressive amount of skin, so dirty plates might get some spots on them.

False Negatives: Tiny crystals beyond what we can resolve will be difficult to focus on, or difficult to distinguish - these could be considered false negatives. Actual false negatives would only occur one of three ways:

- **UV doesn't make it to the crystal.** This could be caused by media absorption, or (less likely) solution absorption. UV might not penetrate into a very, very deep well, which can happen in 5 mL drops.

- **UV makes it to the crystal, is absorbed, but the protein doesn't fluoresce** (no tryptophan, damaged/bleached tryptophan).

- **The fluoresced UV doesn't make it to the camera** (media absorption or solution absorption again).

All three of these scenarios are unlikely, given carefully chosen media and reasonable drop sizes.


*Q:  Where do you place the UV lens?  Does it image from the top of the plate or from the bottom? If from the top, then we will have to put the glass sandwich plates upside down.  Does it affect the imager operation?*

The UV lens is above the plate. Putting the plates in upside down should be fine – you'll just need to switch the barcode to the other side. You may want to try this in your system to make sure there are no unexpected consequences.

# SONICC FAQs

**Background**

***Q:  What is SHG?***

SHG stands for Second Harmonic Generation and is a nonlinear optical process. In intense electric fields (i.e. in the presence of a femtosecond laser) the distance between the electrons and the nucleus are distorted (anharmonicity) resulting in nonlinear optical effects such as SHG where the frequency of the outgoing light is doubled that of the incident (i.e. 1064 nm incident results in 532 nm exiting).

***Q:  What does "chiral" mean?***

A chiral molecule, or in this case a chiral crystal, is a crystal that lacks an internal plane of symmetry, and thus its mirror image is non-superimposable on itself. Achiral crystals are symmetric and therefore produce SHG in equal and opposite directions that sum to a net of zero signal.

***Q:  Will all protein crystals be detectable?***

Almost all molecules that have a chiral center form a chiral crystal, therefore most proteins will form chiral crystals that are detectable via SONICC. The amount of signal generated by protein crystals depends on the symmetry of the crystal and the protein type. Highly symmetrical crystals will generate less SHG. Also, some proteins are not susceptible to SHG and will generate low amounts of signal.

***Q:  Will salts produce signal?***

They can if they are chiral, but the majority of salts are achrial and therefore do not generate SHG.

***Q:  How is SONICC different than fluorescent imaging?***

Fluorescent imaging takes advantage of either the endogenous fluorescence of the protein or the use of fluorescent tags. Although the fluorescence is bright and easily detectable, it is generated from solubilized and aggregated proteins as well as crystallized proteins. The background from the solubilized protein decreases the signal to noise ratio significantly and false positives can result from aggregated proteins. SONICC on the other hand is only sensitive to crystallized proteins.

**Q: How does SONICC compare to UV imaging?**

UV fluorescence probes the amino acids present in proteins that are excited in the UV (~280 nm). It does not differentiate between solubilized, aggregated or crystalline protein. Also, the use of the high energy wavelengths can cause damage to the proteins especially through breakage of disulfide bonds. This does not happen with SONICC because much longer wavelengths are used.

**Q: How does SONICC compare to birefringence imaging?**

For clear birefringent images, crystals usually need to be greater than 30 μm in size, however SONICC can detect crystals smaller than 1 μm. Birefringence can also be seen from salt crystals.

**Q: What platforms is SONICC compatible with?**

All optically assessable platforms are compatible.

**Q: Will the laser damage my crystals?**

Preliminary experiments show no detectable damage to protein crystals. In one experiment, a protein crystal was imaged on one half with excessive laser input. X-ray diffraction was obtained from both the exposed and un-exposed halves of the crystals. Both sides diffracted to within expected resolution (~2 A) and within statistical variation (i.e. there was no statistical difference between the diffraction of both sides). SONICC has also been utilized to image live cells with no observed impact (they remained adhered to a Poly-Lysine coated slide).

**Q: Can I still use SONICC if my sample is fluorescent?**

Yes, as long as the fluorescence is stokes shifted by 10 nm, then it will not be detected or interfere with the SHG.

**Q: Can SONICC be used to detect crystal quality?**

Unfortunately, as of yet it can not, but we are looking into means of assessing quality based on polarization changes of the emitted light.

**Specifications**

*Q: How small of a crystal can SONICC detect?*

Theoretically the lower limit of detection can be estimated by the forward to backward ratio of the SHG. Based on the coherence length of the generated SHG and the refractive index of the material this lower limit ranges from 90 nm – 300 nm in thickness. In practice, crystals of the size of 1 $\mu m^3$ can routinely be detected. 2-D crystals have also been routinely imaged with S/N > 30.

*Q: What is the spatial resolution?*

Dependent on the field of view being imaged, pixel sizes range from 0.5 µm to 3.5 µm. The spot size of the laser is 3.5 µm for the large field of view objective, and 1.5 µm for the highest magnification.

*Q: What is the z resolution and how deep can it penetrate?*

The laser focuses to a width of 50 um – 10 µm (depending ob the objective) and can image drops greater than 3 mm tall with multiple z-steps.

*Q: How fast is SONICC?*

The current electronic package allows for 512 x 512 image acquisition for one z-slice in 500 ms. This corresponds to 8 traces of the fast scanning mirror per line. A one drop 96 well plate can be imaged with SHG in 15 minutes with 8 z-slices and 5 minutes for visible imaging.

# API (Active Pharmaceutical Ingredients) Applications

*Q: Why use SONICC to detect APIs?*

Bioavailability is greatly decreased if the APIs crystallize and is therefore extremely important to monitor. Current methods of crystallization detection (thermal, optical, x-ray diffraction and spectrochemical techniques) have a 1% crystallinity limit of detection and are challenging in complex matrixes. Early crystallization detection expedites the process of monitoring APIs and can also give insights crystallization kinetics.

*Q: What percent crystallinity can SONICC detect?*

The theoretical detection limit, estimated by the coherence length of SHG and refractive index of griseofulvin is 90 nm in thickness corresponding to ~ $3.4 \times 10^{-9}$ % crystallinity. An upper limit of detection would be >5 µm corresponding to % crystallinity of > $6 \times 10^{-4}$% which is 5 orders of magnitude improvement from existing techniques.

**Q**: *How does it compare to conventional techniques?*

Raman, differential scanning calorimetry, and X-ray diffraction routinely have detection limits ranging from 0.1 – 10 % crystallinity.

# FRAP FAQs

**Q: What is FRAP?**

FRAP stands for Fluorescence Recovery after Photobleaching. It is an optical technique that has been broadly adopted in the bioscience community to quantify two dimensional lateral diffusion rates of fluorescently-labeled molecules in a thin film layer.

**Q: What benefit will the LCP-FRAP assay bring to GPCR crystallization?**

Lipid cubic phase (LCP) protein crystallization has proven successful to obtain high resolution structure of the GPCR protein. This method depends on the diffusion ability of the protein in a lipid matrix. Membrane protein diffusion rates are affected by the following parameters: protein aggregation state, the structural parameters of the LCP, and chemical environment. Screening crystallization conditions in this multi-parameter space is a research bottleneck. Automated high throughput LCP-FRAP assay is able to prescreen crystallization conditions and identify positive conditions.

**Q: Is LCP-FRAP integrated with other Formulatrix imaging products?**

FRAP is offered as a standalone system but can also be integrated with the Rock Imager 1000. The Rock Imager 1000 with FRAP maintains two independent imaging stations. You are able to run a FRAP experiment in parallel with bright field color imaging.

**Q: Do I need to run FRAP drop by drop?**

You can opt to run FRAP in a single LCP drop. However. the system is built with a hands-free operation in mind. You only need to set up the FRAP inspection schedule; the system will automatically load the plate and finish the whole FRAP inspection.

**Q:  How are the FRAP results presented to the user?**

Protein mobility results are saved in a central database and presented to the user in a canvas view. Color codes are assigned to each drop to highlight the positive drops.

**Q:  Do I have to dye-label my protein sample?**

Yes. Proteins must be labeled with a fluorescent dye. We suggest Cy3 as the preferred labeling dye.

**Q:  Any special requirements on plates, spacers and cover slips?**

All glass base plates and glass cover slips work very well. However, to achieve accurate and repeatable mobility measurements, we suggest 60 μm spacers to maintain a thin sample thickness. FRAP is measuring a two dimensional molecule diffusion rate. Thin sample thickness will eliminate the diffusion effects in the third dimension. Thin sample thickness will also facilitate high contrast bleaching spot, which leads to more accurate diffusion results.

# Maintenance

*Chapter 15*

You should not open the system for maintenance unless expressly instructed by Formulatrix. Please contact the Formulatrix Support team via email - support@formulatrix.com for any issue encountered during the operation of your Rock Imager 1000. The support email address is monitored 24 hours a day, Monday through Friday; a response will be provided as soon as possible.

- Some computers require greater amounts of time to connect to the Rock Imager, so you may need to wait for up to 10 seconds after turning on the Rock Imager to run the software.

- Make certain that each cable is properly connected to the computer.

- Please feel free to contact Formulatrix via email or phone with any questions. We are always happy to hear from our customers and provide assistance.

- Direct the Rock Imager drain tube to a drain container to hold the water from the cooling units (RI 1000 only).

- Check the drain container periodically and make sure to empty it when the water is full.

## Maintenance Coverage Information

*Q: What is included in software maintenance?*

Software maintenance includes all product upgrades (excluding installation), on-site support to fix problems, phone support, and remote support (via remote connectivity tools like VPN access, WebEx, GoToMyPC.com, etc).

*Q: What is included in hardware maintenance?*

Hardware maintenance includes all parts, labor, on-site support, phone support and remote support (via remote connectivity tools like VPN access, WebEx, GoToMyPC.com, etc) that are required to keep the hardware operational (including monitoring and responding to regular diagnostics).

**Q:  What is not covered under software and hardware maintenance?**

Hardware maintenance does not cover any damage to the system due to abuse of the system, system modifications, or misuse of the system by the customer. Travel and shipping expenses including shipping of defective parts during the maintenance (warranty) period will be billed separately at actual cost. A fixed cost option is available (if desired). Software and hardware maintenance do not include extended user training, they only cover training at the time of hardware installation.

**Q:  Are computers covered under maintenance?**

Computers supplied by Formulatrix are not covered under maintenance. We purchase a minimum 2 years on-site service plan from the computer manufacturer and transfer this warranty to the customer. Formulatrix will assist the customer to diagnose any computer errors and re-install the software, but the customer must work with the computer manufacturer directly to resolve any hardware issues.

**Q:  What is priority shipping and travel?**

You can choose to pay for travel and priority shipping in advance for up to one year. Please ask your sales or support person for a quotation.

**Q:  What happens if I am out of maintenance and I have a problem?**

If you are out of maintenance you can choose to purchase support time at an hourly rate and pay for time and materials for hardware related issues separately.

**Q:  What happens if I am out of maintenance and I want to get back on maintenance?**

You must pay back maintenance to renew. For example, if your maintenance expired in June '10 and you want to renew maintenance in December '10, then you must pay back maintenance to cover the time period of June to December. In this scenario, purchasing 1 year of maintenance will cover you from June '10 to June '11. Any support call purchased during a repayment period will be credited towards your next maintenance payment.

# Apendix A
*Optics Controls*

This table explains how to use each of the optics controls, which are used during manual inspections. See _Manual Inspections_ on page 53 for more information.

| Control | Imaging Methods | Description |
|---|---|---|
| **Zoom** | • Visible<br><br>• UV<br><br>• FRAP<br><br>• SONICC | The Zoom field populates with the current zoom setting and is related to the FOV field. These fields allow you to zoom in on regions of interest within a drop or well.<br><br>Available zoom settings depend on the options you purchased. In most cases the zoom selection is a physical change of the objectives.<br><br>In SONICC, changing the zoom value changes the laser beam scan angle. |
| **Field of View (FOV)** | • Visible<br><br>• UV<br><br>• FRAP<br><br>• SONICC | You can use the FOV fields to manually set the size, in µm, of the image you would like to view. As you change the FOV value using either the text entry field or the arrows, the zoom value automatically adjusts. |
| **Bright Field** | • Visible<br><br>• FRAP | Bright field illumination passes light through the specimen under the microscope so that the specimen appears dark against a bright background. At 0%, the bright field light is turned off, and at 100% the light is at maximum brightness. |

*This table explains how to use each of the optics controls, which are used during manual inspections. See Manual Inspections on page 53 for more information.*

| Control | Imaging Methods | Description |
|---|---|---|
| **Condenser** | • Visible<br><br>• FRAP | The condenser collects light from the Köhler light source and concentrates it onto the well being examined. Values range from 0 – 100%. At 0%, the iris is fully open, and a cone of light is concentrated at a wide angle. At 100%, the iris is closed, and the light is concentrated in a column of light as opposed to a cone.<br><br>Adjusting the condenser value can improve image contrast. Increasing the condenser angle increases the contrast. |
| **Polarizer** | • Visible | This field describes the angle at which the polarizer lens is projecting the light from 0 - 360°. Recommended settings are 0° or 90°. At 0°, you are not using cross polarized imaging. At 90°, you are using cross-polarized imaging. At 90°, if there is a crystal in the FOV, the crystal will light up due to the crystal's birefringence (if there is no crystal, the FOV will appear dark). |
| **Illumination** | • Visible<br><br>• UV<br><br>• SLP UV | On the visible imaging method, the illumination list provides you with various illumination patterns, including glancing, dark field, and bright field illumination. With the UV imaging method, the illumination button controls whether the LED UV light source is on or off. |
| **Laser** | • FRAP | Controls whether the laser, which bleaches the sample, is on or off. |
| **Objective** | • SONICC | The Objective control offers 10x, 20x or 40x magnification through the drop-down list. Your selection affects Zoom and FOV options. |

*This table explains how to use each of the optics controls, which are used during manual inspections. See Manual Inspections on page 53 for more information.*

| Control | Imaging Methods | Description |
|---|---|---|
| **Sensor** | • SONICC | The Sensor selection determines whether you are using SHG or UV-TPEF.<br><br>The SHG imaging method images the sample with IR and detects the SHG (532 nm) in transmission. Signal is only generated from chiral crystals.<br><br>The UV-TPEF imaging method images the sample with green light (532 nm) and detects the two photon excited fluorescence generated (350-400 nm). Signal is generated from UV-fluorescent molecules like tryptophan. |
| **Fluoro Light** | • FRAP | The fluoro light is the florescent light source. Changing this value affects the fluorescence signal intensity. 100% is the most intense. |

# Appendix B
*Camera Controls*

*This table explains how to use each of the camera controls, which are used during manual inspections. See Manual Inspections on page 53 for more information.*

| Control | Imaging Methods | Description |
|---|---|---|
| **Live Image Radio Button** | • Visible<br>• UV<br>• FRAP<br>• SONICC | Turns the Live Image on and off.<br><br>The longer you keep a single drop under a live image, the more you subject your sample to heat, so we recommend turning the live image off when not actively inspecting wells.<br><br>In SONICC, the first image will be mistimed, but can be reset by turning Live Image OFF and then ON again. |
| **Exposure** | • Visible<br>• UV<br>• FRAP<br>• SONICC | The Exposure field populates with the amount of time in milliseconds that the camera's shutter remains open when capturing an image, which determines the amount of light allowed to fall on the camera's sensor affecting the image's brightness.<br><br>Clicking the Auto button runs the Auto Exposure algorithm (Visible and FRAP only). |
| **Resolution** | • Visible<br>• UV<br>• FRAP<br>• SONICC | The resolution of an image is the size of the image in megapixels.<br><br>Drop-down options depend on the camera you purchased. |
| **Leveling** | • Visible<br>• UV<br>• FRAP<br>• SONICC | Clicking the Leveling button will open the Leveling Histogram. The Leveling Histogram is used to adjust image brightness and contrast (see Leveling Histogram for further details).<br><br>If you adjust these settings, it only affects the way the Live Image displays, and not the way the image is captured. |

*This table explains how to use each of the camera controls, which are used during manual inspections. See Manual Inspections on page 53 for more information.*

| Control | Imaging Methods | Description |
|---|---|---|
| **Auto Focus** | <ul><li>Visible</li><li>FRAP</li></ul> | Clicking the Auto Focus button executes the Auto Focus algorithm. |
| **Locate Drop** | <ul><li>Visible</li><li>FRAP</li></ul> | Clicking the Locate Drop button executes the Drop Location algorithm on the drop currently in view. This algorithm analyzes the drop's well to determine the exact location of the drop and displays the results on the Drop Location tab.<br><br>This button is only available if Drop Location has been enabled for the plate type you are using, which is controlled through the Plate Type Editor. |
| **Gain** | <ul><li>Visible</li><li>UV</li><li>FRAP</li></ul> | Used to amplify signal strength in low-light conditions, increase the sensitivity and make the image brighter.<br><br>Increasing the amount of gain can also increase the noise in an image. |
| **Gamma** | <ul><li>Visible</li><li>UV</li></ul> | This field affects the contrast of the midtones in the image.<br><br>When you modify this setting, only the midtones are affected, without affecting black and white. Changing this setting affects both the Live View and automatic imaging. |

*This table explains how to use each of the camera controls, which are used during manual inspections. See Manual Inspections on page 53 for more information.*

| Control | Imaging Methods | Description |
|---------|-----------------|-------------|
| Laser | • SONICC | This field affects the laser power at the sample. The On and Off radio buttons turn the Laser on and off. There is a percentage column to interpret the mW value of laser power to the percentage form.<br><br>Signal strength from both SHG and UV-TPEF imaging methods scales with the quadratic of the incident laser power. There is a large dynamic range of signal intensity from one sample. It is best to test a few drops with SONICC in manual mode to determine the optimal laser power.<br><br>**UV-TPEF:** The signal intensity is dependent on the number of tryptophans in the protein. A good starting point would be at about 50% of the laser power available.<br><br>**SHG:** The signal intensity is dependent on the presence or absence of a chromophore, crystal class and quality. For example, proteins with chromophores need only ~100 mW whereas 350 mW would be an appropriate power for other samples. A good starting point is 300 mW and then adjust according to your specific sample. The maximum laser power is ~550 mW (dependent on system), but take caution when using such high powers for extended periods on samples. Routine imaging with < 400 mW is optimal. |

# Index