Rock Imager 1000 SONICC How it Works & FAQ

<http://www.formulatrix.com/demosite/protein-crystallization/products/rock-imager-1000/index.html#tabbed-nav=tab5&tabbed-nav-sonicc=tab2>

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**Advanced Overview of How SONICC Works**

Second Order Nonlinear Imaging of Chiral Crystals (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 (Figure 1). This process only occurs in non-centrosymmetric 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.

[Read More](http://www.formulatrix.com/demosite/protein-crystallization/products/sonicc/how.html)

**SONICC Frequently Asked Questions**

I. Background

**1. 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).

**2. 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 nonsuperimposable. Achiral crystals are symmetric and therefore produce SHG in equal and opposite directions that sum to a net zero signal.

**3. Are all protein crystals 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. Over 99% of the proteins in the PDB have a space group that is detected by SONICC. Those crystals with extremely high symmetry classes will generate less SHG.

**4. Will salts produce signal?**

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

**5. 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 S/N significantly and false positives can result from aggregated proteins. SONICC on the other hand is only sensitive to crystallized proteins.

**6. 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.

**7. 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 down to less than 1 µm. Birefringence can also be seen from salt crystals.

**8. With which platforms are SONICC compatible?**

SONICC is compatible with all optically assessable platforms.

**9. Can I do TPEF (Two Photon Excited Fluorescence) at the same time?**

With the current setup the TPEF can be detected, but not simultaneously. The next version of the instrument will permit multiple channels of detection simultaneously.

**10. 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 Å) 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).

**11. 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 nor interfere with the SHG.

**12. Can it be used to detect crystal quality?**

Unfortunately, as of yet it cannot, but we are investigating ways of assessing quality based on polarization changes to the emitted light.

II. Specifications

\*Please note that each crystal will generate different intensities of SHG depending on size, orientation, space group and quality as well as the acquisition time and incident intensity.

**1. How small of a crystal can it 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, 1 µm3 crystals can be detected routinely. 2-D crystals have also been routinely imaged with S/N >30.

**2. What is the spatial resolution?**

Dependent on the field of view being imaged, pixel sizes range from 3 µm to 6 µm.

**3. What is the z resolution and how deeply can it penetrate?**

The laser focuses to a width of ~100 µm and can image drops >3 mm tall with multiple z-steps.

**4. How fast is it?**

The current electronic package allows 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.