SONICC FAQ

http://www.formulatrix.com/demosite/protein-crystallization/products/sonicc/index.html#tabbed-nav=tab4

**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 electrons and their nucleus are distorted (anharmonicity) resulting in nonlinear optical effects such as SHG where the frequency of the outgoing light is double that of the incident (e.g. 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 on itself. Achiral crystals are symmetric and therefore produce SHG in equal and opposite directions that sum to a net zero signal.

**3. Will all protein crystals be detectable?**

Almost all molecules that have a chiral center form a chiral crystal. Therefore most proteins form chiral crystals that are detectable via SONICC. Over 99% of the proteins in the PDB have a space group detectable by SONICC. Crystals with extremely high symmetry classes 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 a 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 sensitive only 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 birefringent imaging?**

For clear birefringent images, crystals usually need to be greater than 30 μm. However SONICC can detect < 1 μm. Birefringence can also be produced by 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. Our next SONICC version will have multiple channels and allow simultaneous detection.

**10. Will the laser damage my crystals?**

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

**11. Can I still use SONICC if my sample is fluorescent?**

Yes, as long as the fluorescence is Stokes-shifted by 10 nmit will not be detected nor interfere with the SHG.

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

Not presently, but we are investigating methods of assessing quality based on polarization changes to the emitted light.

II. Specifications:

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

**1. 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, the 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 at S/N > 30.

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

Dependending upon 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 SONICC 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 SONICC?**

SONICC’s electronic package allows 512 x 512 image acquisition per z-slice in 500 ms. This corresponds to 8 traces of the fast scanning mirror per line. A single drop 96 well plate can be imaged in only 3.5 minutes with visible light, and in 15 minutes with 8 z-slices using SHG.