SONICC How it Works

<http://www.formulatrix.com/demosite/protein-crystallization/products/sonicc/index.html#tabbed-nav=tab3>

**Second Order Nonlinear Imaging of Chiral Crystals (SONICC)**

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 noncentrosymmetric ordered crystals. Thus a 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 necessitating the use of a femtosecond laser. The laser operates with a pulse width of 200 fs and has high peak powers resulting in nonlinear effects but in short enough duration to reduce damage associated with localized heating. The laser quickly scans the sample with a resonant mirror and galvanometer-driven mirror to build a 2D image. The SHG signal is then separated from the fundamental by dichroics and filters and detected with a photomultiplier tube (PMT).

The high contrast images obtained with SONICC are due to the specificity for noncentrosymmetric (chiral) crystals. Chiral crystals lack an internal plane of symmetry, and thus its mirror image is nonsuperimposable on itself. Only those samples that lack inversion symmetry will produce a signal. Over 98% of the proteins deposited in the databank are chiral, with 80% of them in a low symmetry class that easily generates SHG. Most salt crystals are symmetric and therefore generate no SHG. Also, all aggregated and solubilized protein will produce no SHG yielding no background signal, making it very easy to reveal hidden crystals.

The images generated by SONICC set it apart from conventional imaging techniques such as fluorescence and birefringent imaging. Whether probing fluorescent tags or tryptophan, fluorescence imaging generates large background signals due to solubilized or aggregated protein.  Fluorescent and birefringent imaging are also limited to larger crystals (>10 µm) making it difficult to locate conditions that generate crystal showers. SONICC detects crystals <1 µm allowing the identification of many more conditions that can be optimized for protein crystal growth.

## UV-TPEF Enables You to Distinguish Between Protein and Salt Crystals

Some salt crystals including lithium sulfate, ammonium sulfate and ammonium phosphate form noncentrosymmetric crystals and produce SHG. The use of UltraViolet – Two Photon Excited Fluorescence (UV-TPEF) allows for the clear discrimination between protein and salt crystals.  This imaging mode takes advantage of the fluorescent aromatic amino acids (e.g. tryptophan) in proteins. When excited with UV radiation (~270 nm) 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 imaging laser in SONICC is coupled with a NLO (nonlinear optical) crystal from 1064 nm to 532 nm. Green light (532 nm) is then used to image the sample (Figure 2). The two photon equivalent of the green source is 266 nm which excites any tryptophan amino acids that are present. The two photon excited fluorescence (325 – 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 are complementary. The SHG channel probes crystallinity while the UV-TPEF channel is specific to proteinaceous samples.

Figure 3 shows that the use of the UV-TPEF channel provides a clear assessment of each sitting drop imaged in the plate. In rows (a) and (b) contrast is observed in both the UV-TPEF and SHG channels 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.

## SONICC for Microcrystal Detection

SONICC can detect crystals <400 nm and therefore is well suited for imaging protein crystals that are not easily imaged with conventional techniques. Recent advances in nanocrystallography by Fromme at Arizona State University have demonstrated the need for an alternate technique to characterize protein samples containing sub-micron crystals. In collaboration with Ross at ASU, they have used SONICC to image protein crystals in microfluidic channels (Figure 4).

The detection limit of SONICC is proportional to the N.A. (numerical aperture) of the objective used and is important to consider when detecting small crystals. SONICC’s optional compound zoom feature allows users to automatically switch objectives depending on the application. For wide field of view (FOV) imaging in traditional sitting drops, the standard asphere included is the optimal solution due to its large working distance, wide FOV, and reasonable N.A. However, for applications such as LCP where crystals formed are relatively small, higher N.A. objectives are more suitable. A comparison of objectives offered can be found here.

## Crystal Class and Expected SHG

The intensity of SHG depends on many parameters including the type of protein, quality, symmetry and orientation of the crystal. In general, as the symmetry of the crystal space group increases, the intensity of SHG decreases. Table 1 lists all space groups in order of increasing symmetry with an analysis of whether it would generate SHG and the percentage of protein structures known in that crystal class. For those higher symmetry crystal classes that are difficult to detect with SHG, one can use UV-TPEF for imaging. The percentage of those proteins that do not contain any tryptophan, and therefore would not be detectable with UV-TPEF, are also included in Table 1.

In general, the following can be concluded:

* 22% are of higher symmetry that may not be possible to detect with SHG
* 16% do not contain any tryptophan and would not be visible in UV-TPEF
* 3.5% are of high symmetry and do not contain tryptophan making them difficult to detect with UV-TPEF or SHG

Preliminary experiments show no detectable damage to protein crystals due to 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 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. The cells remained adhered to a polylysine coated slide before, during and after imaging indicating they remained viable.

NOTE: Change the label of ‘Figure 5’ to Table 1