Coherent Anti-Stokes Raman Scattering Imaging: A Basic Overview with an Emphasis on the Optics Required

Coherent anti-Stokes Raman scattering (CARS) imaging is a nonlinear optical technique that captures intrinsic molecular vibrations to create optical contrast. It offers submicron level spatial resolution and video-speed imaging rate without the needs for extrinsic labels (e.g. fluorochromes). In a CARS process, a pump laser, a Stokes laser and a probe laser (usually same as the Pump laser) are involved. When the frequency difference (beating frequency) between the pump and the Stokes lasers is in resonance with the frequency of a molecular vibration, an enhanced optical signal is generated at the anti-Stokes frequency. The major advantage of CARS is that the signal yield is much higher, typically six orders of magnitude, than the signal yield obtained through the conventional spontaneous Raman scattering process. By tuning the beating frequency to a designated vibrational mode of a chemical bond (e.g. O-P-O, H-C-H), CARS enables chemically selective imaging of targeted molecules. Several optical designs have been reported for CARS imaging. These designs use different wavelength combinations for the pump and Stokes beams, resulting in variations of CARS emission wavelength for the same chemical bond. Consequently, different dichroic mirrors and emission filters are required. In this application note, we will primarily focus on filter sets for CARS excitation of symmetric vibration of the H-C-H bond, which is the most popular vibrational mode used in biomedical imaging applications.

The first configuration utilizes a pair of Ti:sapphire lasers (e.g. 5-ps, Tsunami, Spectra-physics) as the pump and the Stokes lasers. In this configuration, an output of 700 to 840 nm from one laser is used as the pump while an output of 782 to 900 nm from the other laser system is used as the Stokes. Both shortpass and longpass dichroic can be used depending on the system configuration. For CH_2 imaging, typically recommended filters are as follows:

Dichroic (Fig. 1): Shortpass: 680dcspxr (lot 203184)/zt700dcspxxr (lot 209488); Longpass: 670dcxxr (lot 208561)/700dcxxr (lot 212919). **Emission Filter** (Fig. 2): ET680sp-2P8 (lot 238150), ET700sp-2P8 (lot 237176), ET605/70m-2p (lot 202293)

Note: Dichroic mirrors are designed to be used at 45° AOI while emission filters are designed to be used at 0-5° AOI. All lot numbers are listed for illustration purpose only.

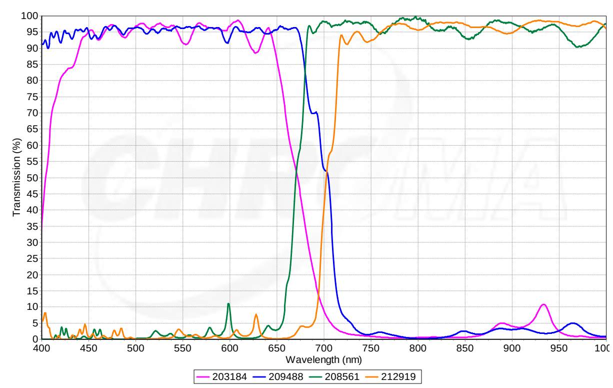

Figure 1: Transmission diagrams of the dichroic mirrors for the first CARS configuration which utilizes a pair of Ti:sapphire lasers. For wavelengths from visible to NIR, a reflection diagram can be calculated by inversing a transmission diagram assuming zero absorption.

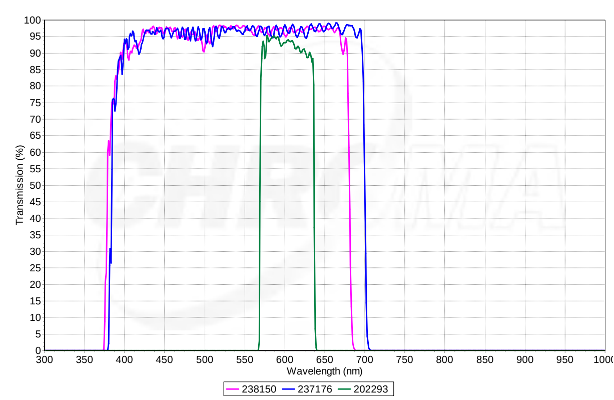

Figure 2: Transmission diagrams of the emission filters for the first CARS configuration.

For CARS imaging, we recommend the use of a serial of two emission filters before the detector for optimal blocking (theoretically to OD10) of the excitation lasers (Fig. 3). A combination of a shortpass filter (ET680sp-2P8 or ET700sp-2P8) with a bandpass filter (e.g. ET605/70m-2p) is recommended to serve this purpose. ET680sp-2P8 and ET700sp-2P8 are specially designed filters with an average OD8 blocking up to 1060 nm. Two-photon filters are recommended over one-photon filters as potential bandpass filters in combination with ET680sp-2P8 or ET700sp-2P8. This is because of their additional blocking (an average of OD6) in the NIR region. For example, both ET660/30m (lot 215558) and ET740/60m (lot 242560) might satisfy the spectral requirement of certain CARS emission. However, both of them possess a certain level of spectral leakage around 900-1000 nm (Fig. 4). This leakage might allow a small portion of excitation lasers to enter the detector, resulting in a lower S/N ratio. In contrast, ET660/40m-2p (lot 211696) and ET750/50m-2p (lot 215534) are better options to cover similar bandwidths by holding an average of OD6 blocking across NIR (Fig. 5). Still, under certain circumstances, some of the one photon filters (e.g. ET731/30m, lot 19731), which also block beyond 800 nm, could be utilized, especially when being used in combination with the OD8 blocking shortpass filters.

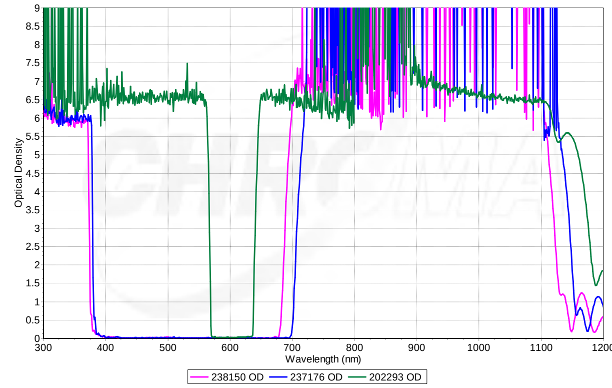

Figure 3: OD diagrams of the emission filters for the first CARS configuration.

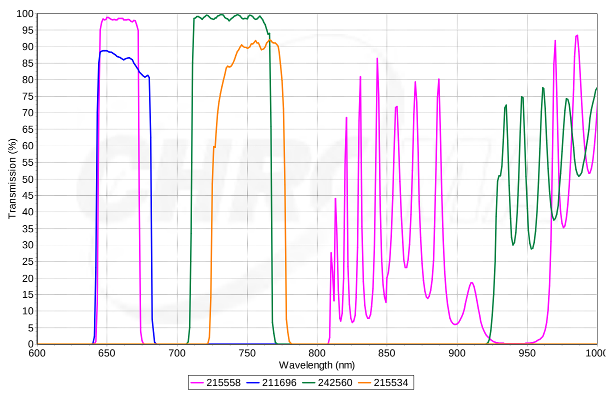

Figure 4: Transmission diagrams of representative one-photon and two-photon bandpass emission filters.

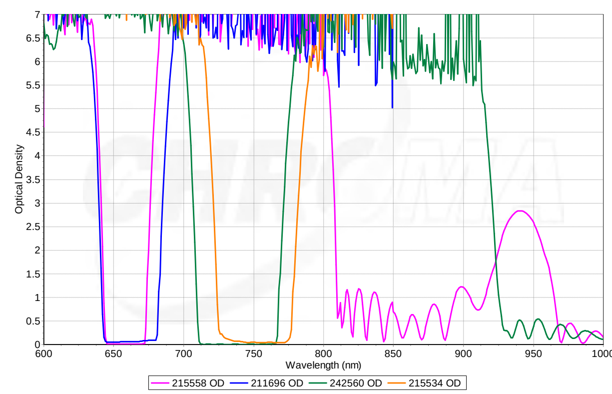

Figure 5: OD diagrams of representative one-photon and two-photon bandpass emission filters.

The second configuration includes a frequency-doubled Nd:YVO4 modelocked laser (e.g. High-Q) and an optical parametric oscillator (OPO). The Nd:YVO4 laser delivers 7-ps, 76-MHz pulse trains at both 532 nm and 1,064 nm wavelengths. The 532 nm beam is used to pump the OPO, which generates two 5-ps tunable outputs (called signal and idler): a signal beam from 670 nm to 980 nm and an idler beam from 1130 nm to 1600 nm. A zero degree longpass filter (e.g. ET650lp, lot 215145) is normally used right after the OPO to block the residual 532 nm pump laser (Fig. 6).

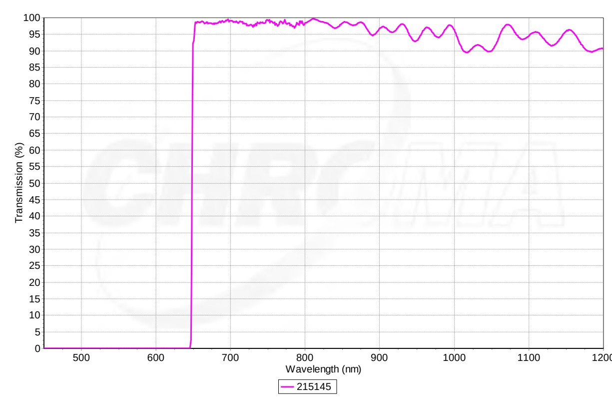

Figure 6: Transmission diagrams of a representative longpass filter for blocking of the 532 nm pump laser after the OPO.

Two types of strategies have been reported in using these beams for CARS excitation. (1) To use the signal beam from OPO as the pump and use the 1064 nm output from the Nd:YVO4 laser as the Stokes. (2) To use the signal and idler beams from OPO as the pump and the Stokes, respectively. For scenario (1), an additional dichroic beam combiner (BCM) is needed to combine the signal beam and the 1064nm beam. Depending on the system configuration, either a shortpass dichroic or a longpass dichroic can be used to serve this purpose. Bellow is our recommended filter set.

(1) Pump: Signal from OPO: 775-920 nm, Stokes: Nd:YVO4 modelocked laser (1064 nm)

BCM (Fig. 7): T970dcspxr (lot 226962), q1020lpxr (lot 212733)

Dichroic (Fig. 8)**:** Q770dcxxr (lot 211623), 750dcxrxt (lot 204792)

Emission Filter (Fig. 8)**:** ET680sp-2P8 (lot 200185), ET700sp-2P8 (lot 200101), ET720sp-2P8 (lot 204561), ET750sp-2P8 (lot 212882), ET660/40m-2p (lot 211696)

(2) Pump: Signal from OPO: 670 nm to 980, Stoke: Idler from OPO: 1130 nm and 1600 nm, e.g. P: 924 nm, S: 1254 nm

Dichroic (Fig. 9)**:** Q770dcxxr (lot 211623)

Emission Filter (Fig. 9)**:** HQ750/210m-2p (lot 226179), + ET731/30m (lot 219534)

It is worth mentioning that HQ750/210m-2p is a very useful emission filter for CARS imaging because of its superior blocking capability of excitation lasers to an average of OD6 in NIR (Fig. 10). Although the transmission range of this filter has extended into the transmission range of the dichroic mirror, HQ750/210m-2p mainly functions to block the excitation lasers instead of selection of designated emissions.

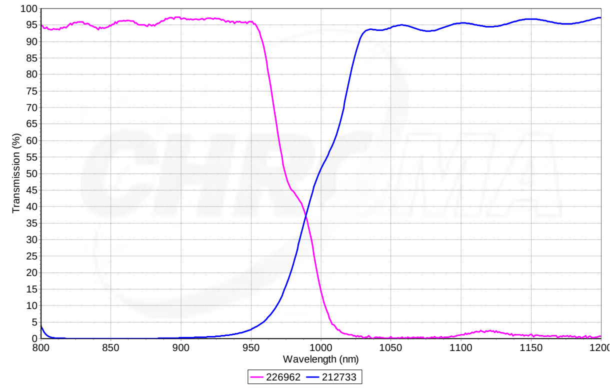

Figure 7: Transmission diagrams of dichroic mirrors for combination of the pump and Stokes beams.

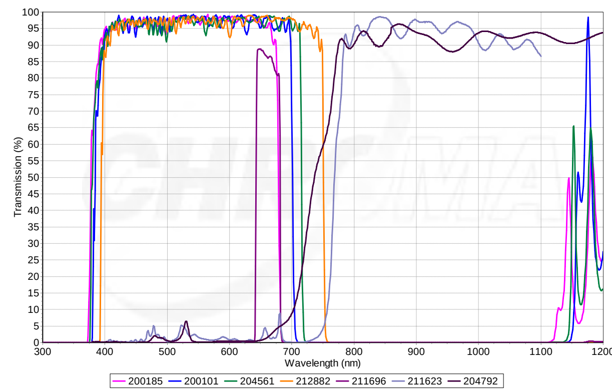

Figure 8: Transmission diagrams of the microscopy dichroic mirror and emission filters for scenario (1) in the second configuration for CARS imaging.

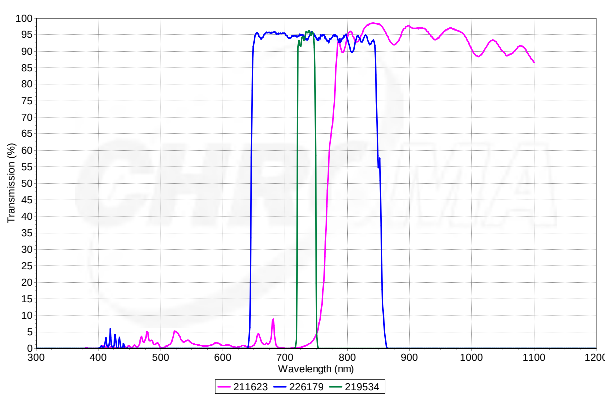

Figure 9: Transmission diagrams of the microscopy dichroic and emission filters for scenario (2) in the second configuration for CARS imaging.

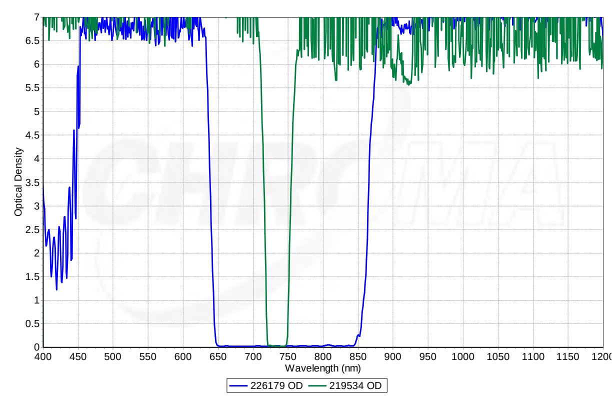

Figure 10: Transmission diagrams of the microscopy dichroic and emission filters for the third system configuration.

The third configuration utilizes a picosecond Nd:Vanadate laser pumping two OPOs. Two of the three beams (The two signal beams from the OPOs and the 1064 nm laser) can be used as the pump and Stokes beams, enabling probing any molecular vibration in the important Raman fingerprint range of 0-3,400 cm^{-1}. For CH_2 imaging, typically recommended filters are:

Dichroic (Fig. 11)**:** 750dcxrxt (lot 204792)

Emission Filter (Fig. 11)**:** ET680sp-2P8 (lot 200185), ET700sp-2P8 (lot 200101), ET720sp-2P8 (lot 204561), ET580/30m-2p (lot 234074), ET605/75m-2p (202293) or ET660/40m-2p (lot 211696)

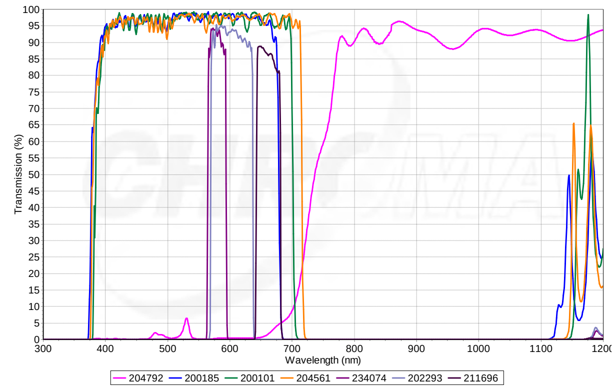

Figure 11: Transmission diagrams of the microscopy dichroic mirror and emission filters for scenario (3) in the second configuration for CARS imaging.

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