**Detector CV macro**

This macro calculates the coefficient of variation (CV, ratio of standard deviation to mean) of the intensity in the confocal image acquired in standardized conditions. CV is a good indicator of the overall sensitivity of the confocal detector and can be used for characterizing and comparing detectors inside one and across different confocal systems and specific spectral ranges. The detector with highest sensitivity has lowest CV value. The input for macro is two consecutive images, detailed measurement protocol is below.

This macro is inspired by work of Zucker: R.M. Zucker and O.Price, Cytometry 44:273–294 (2001). Big thanks to Kota Miura for helpful suggestions regarding the macro programming.

**Measurement protocol**

1. Take the objective of choice and set the laser power in the sample plane to a given value in the range of 5 - 20 W.

2. Use stable uniformly stained sample with fluorescence in the spectral range of interest (e.g. Chroma calibration slide or large beads). Always do the measurement at the same sample depth.

3. Set up imaging conditions as following:

image bit depth – 8 bit; unidirectional scan; image size - 512x 512 pixels; pixel size - 50 nm; pinhole - 1 AU; pixel dwell time - 2.5 s.

4. Adjust PMT detector gain to have a mean pixel intensity about 70 % of detector range (about 180 out of 256 (8 bit)).

5. Take two consecutive images in the given conditions.

6. Use the above data as input for the macro.

7. Macro measures the CV value of the pixel intensity distribution for given detector.

8. Repeat the measurement for another detector, spectral range, confocal system or time point if necessary.

9. The detector with the smallest CV value will be the most sensitive detector.