As announced, the focus of this exam is fluorescence correlation spectroscopy (FCS), specifically its principles and analytical applications. The first half (approximately) of the questions refer to the FCS technique in general. The second half refer specifically to "Simultaneous two-photon excitation of distinct labels for dual-color fluorescence crosscorrelation analysis," Heinze K.G., Koltermann A., Schwille P., *PNAS* 97: (19) 10377-10382 (2000). A copy of this article is attached for your reference. Read the article and answer the following questions in the blue book. Do not write your name on the blue book; write your name on the enclosed index card and put the card in the envelope on the front desk.

1. (10 pts) Explain the fundamental principle of fluorescence correlation spectroscopy. Explain what is measured and what information can be obtained from the measurement. Contrast this measurement to 'the usual way' of measuring fluorescence.

2. (20 pts) Different instrument configurations can been used to measure FCS. Most (like the one described by Heinze et al.) are based on confocal microscopes. Draw a schematic diagram of a FCS instrument that uses totally internally reflected excitation. Show the path of all light beams. Label all the major components as specifically as possible. Explain the nature and source of the particular advantages and limitations of this arrangement?

3. (10 pts) FCS is a 'hot topic'. When a topic is hot, its limitations can be easy to ignore, but they are still there. Describe as many limitations of FCS as you can.

The following questions refer to the paper by Heinze et al.

4. (10 pts) Summarize the work described by the article. What is the objective? Describe the chemical system under observation. Precisely what was done?

5. (10 pts) Describe 2-photon excitation and explain what particular advantages 2-photon excitation brings to fluorescence correlation spectroscopy.

6. (15 pts) Distinguish the fluorescence autocorrelation from the cross-correlation. You may use equations if you choose, but your discussion should define each term conceptually rather than mathematically. Explain what information one wants from each (and both) data processing regime.

7. (15 pts) Explain how the data in Figure 6 were acquired. What is the significance of these results, i.e. what limitations of the dual color method are revealed by this data?

8. (10 pts) The authors propose two photon excitation as a means to facilitate simultaneous investigations of multiple fluorescent species on the single molecule scale. Discuss this assertion. Agree or disagree as you see fit, but discuss the evidence that is true. Discuss any obstacles to 'simultaneous investigations' that must be addressed including the limitations of the analysis described in the paper.