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DEPARTMENT OF BIOSCIENCES AND BIOENGINEERING
 INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
 BT 624: FLUORESCENCE TECHNIQUES IN BIOTECHNOLOGY
 Quiz-II (April 12, 2024)

Total marks = 10

Time = 30 minutes

Name: _____

Roll N. _____

Instructions:

- (a) The question paper contains 9 questions.
- (b) Attempt all questions.
- (c) Q. Nos. 1-8 carry 1 mark each.
- (d) Q. 9 carries 2 marks.
- (e) Each wrong answer in Q. No. 2 will invite a penalty of 0.5 marks.

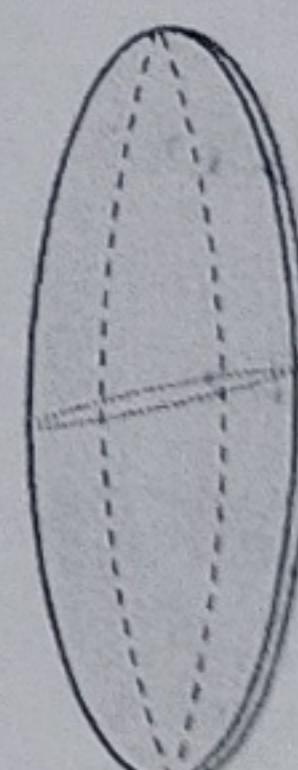
1. In fluorescence correlation spectroscopy, the amplitude of the autocorrelation function $G(0)$ is inversely proportional to the average number of fluorescent particles within the detection volume. Fill in the correct answer from these three options: {1 mark}

- i. directly proportional to
- ii. inversely proportional to
- iii. independent of

2. Which of the following techniques does not/do not need a pinhole to reject the out of focus light? {1 mark: -0.5 mark for each incorrect answer}

- (a) Laser scanning confocal microscopy
- (b) Multiphoton fluorescence microscopy
- (c) Total internal reflection fluorescence microscopy

3. Suppose a lens has different curvatures along two orthogonal directions (shown by dotted and dashed curves in the figure on right). The aberration caused due to this mismatched curvature is known as astigmatism {1 mark}



4. Name any one super-resolution fluorescence microscopic method. {1 mark}

STED microscopy
 (Stimulated emission depletion)

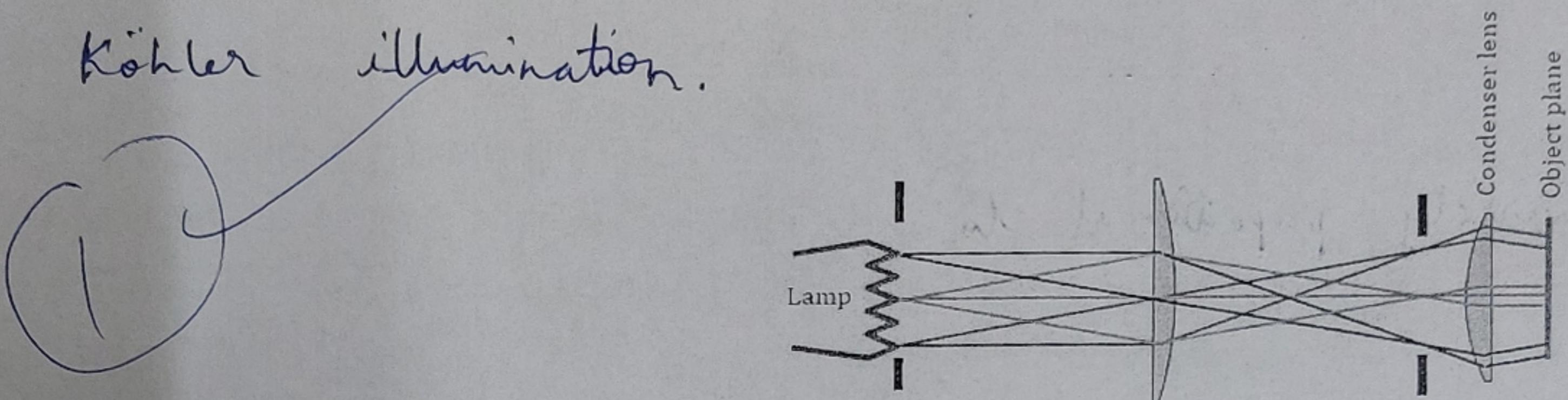
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5. What is the role of tube lens in the infinity conjugate imaging? {1 mark}

Focuses parallel rays to the focus, so that an image is formed. Optical elements such as polarizers can be placed before the tube lens in infinity conjugate imaging as the tube lens will focus the parallel light rays.

6. What kind of specimen illumination is shown in the diagram below? {1 mark}

Köhler illumination.



7. Name any one fluorescence-based method that can be used to study the binding of a fluorescent small molecule to a non-fluorescent macromolecule. {1 mark}

Fluorescence correlation spectroscopy (FCS).

8. What is the role of a dichroic mirror in a filter cube that is used in epifluorescence microscopy?

A dichroic mirror will allow (transmit) {1 mark}

certain wavelengths through it and reflect certain other wavelengths (which can be used to excite the fluorophore).

9. Determine the Abbe's resolution limit for the following set-up of the objective lens when light of 420 nm is used for imaging (Given: the numerical aperture of the condenser lens is higher than that of the objective lens). {2 marks}

Since N.A. of condenser lens is higher, limiting factor for resolution is the N.A. of objective lens. $\Rightarrow d_{\min} = \frac{1}{NA} = \frac{1}{n \sin \theta} = \frac{420}{\sin(30^\circ)} = 840 \text{ nm} = 0.84 \mu\text{m}$

