# Grading of Problem Set #6: Fluorescence Microscopy in Living Cells

Lecture "Interactions of Proteins and Nucleic Acids: Biophysical Concepts and Theoretical Descriptions (BPC2024)"

Winter Term 2024/2025 | Prof. Dr. Karsten Rippe

Web page: [https://malone.bioquant.uni-heidelberg.de/teaching/BPC\_lectures/BPC\_1+2.html](https://malone.bioquant.uni-heidelberg.de/teaching/BPC_lectures/BPC_1%2B2.html)

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## Grading Rubric (per question)

**Note**: The distribution of the maximum point value per question part is given below. Full points can be awarded despite solutions not being perfect.

**11.0-12.0: Excellent**

* Complete, correct methodology
* Clear reasoning
* Independent analysis demonstrated
* Proper units
* **Note 1 to the grader**: Be lenient on minor calculation errors
* **Note 2 to the grader**: Give full points for correct calculations even if explanations are brief

**8.0-10.75: Very Good**

* Minor gaps or errors
* Good reasoning shown
* Some improvements possible
* Mostly independent work

**5.5-7.75: Good**

* Significant gaps present
* Basic understanding shown
* Major improvements needed
* Partial independence

**3.0-5.25: Fair**

* Major conceptual errors
* Some correct elements
* Substantial revision needed
* Limited independent thought

**0-2.75: Insufficient**

* Missing or minimal correct content
* Major misunderstandings
* No demonstrated understanding
* No independent analysis

**Common Point Deductions**

* Missing units: -0.5 per instance
* Unexplained assumptions: -0.5 per instance
* Calculation errors: -0.25 to -1 depending on impact
* Missing references: -0.5 per required citation
* Unclear reasoning: -1 to -2 per section

##

## Distribution of points between question parts for Problem Set #6

**General principles used for Problem Set #6**

* Clear understanding of fluorescence microscopy principles
* Correct analysis of fluorescent protein properties and applications
* Proper interpretation of FRAP and FCS experimental data
* Understanding of phase separation phenomena in biological contexts
* Integration and reference of lecture material and literature
* Please indicate in a comment if unreferenced use of AI tools is suspected

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### Question 1: Autofluorescent Proteins (12 points total)

**1a) 4 points - Selection of single-color fluorophore**

* 2 points: Well-justified selection of appropriate fluorophore
* 1 point: Comprehensive comparison of relevant fluorescent properties
* 1 point: Discussion of advantages for single-color experiments

**1b) 4 points - Multi-color experimental design**

* 1.5 points: Appropriate selection of excitation/emission wavelengths for all four fluorophores
* 1.5 points: Thorough analysis of potential problems (spectral overlap, FRET, photobleaching)
* 1 point: Discussion of technical solutions or workarounds

**1c) 4 points - Protein interaction studies**

* 2 points: Selection of suitable fluorophore pair with clear justification
* 1.5 points: Detailed explanation of selection criteria (spectral separation, brightness, etc.)
* 0.5 points: Discussion of applications and limitations for interaction studies

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### Question 2: FRAP and FCS Experiments with MeCP2 (12 points total)

**2a) 4 points - Types of chromatin interactions**

* 1.5 points: Identification of all interaction modes (stable binding, transient binding, free diffusion)
* 1.5 points: Proper interpretation of FRAP and FCS data for each interaction
* 1 point: Integration of data into a coherent model of MeCP2-chromatin dynamics

**2b) 4 points - Diffusion coefficient detection limit**

* 2 points: Correct derivation or formula for FRAP detection limit
* 1.5 points: Accurate calculation with appropriate parameters
* 0.5 points: Discussion of limitations and technical considerations

**2c) 4 points - FRAP vs. FCS detection capabilities**

* 1.5 points: Identification of interactions detected only by FRAP
* 2 points: Clear explanation of why these interactions are not detected by FCS
* 0.5 points: Discussion of complementary nature of the techniques

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### Question 3: HP1 and Liquid-Liquid Phase Separation (12 points total)

**3a) 4 points - HP1 concentration comparison**

* 1.5 points: Accurate estimation of HP1 concentration in chromocenters
* 1.5 points: Reasonable estimation of HP1 concentration in liquid droplets
* 1 point: Discussion of the significance of concentration differences

**3b) 4 points - HP1-nucleosome stoichiometry**

* 1.5 points: Analysis of HP1-nucleosome binding modes
* 1.5 points: Estimation of stoichiometry within liquid droplets
* 1 point: Comparison with expected stoichiometry in chromocenters

**3c) 4 points - Size and mechanistic comparison**

* 1 point: Accurate size comparison between chromocenters and liquid droplets
* 1.5 points: Analysis of organizing principles for chromocenters vs. liquid droplets
* 1.5 points: Well-reasoned conclusion about chromocenter formation mechanism

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## Point Deductions

* -0.25 points: Minor omissions or unclear explanations
* -0.5 points: Missing references where needed
* -0.75 points: Significant gaps in explanation
* -1 point: Major conceptual errors