UNIVERSITÀ DEGLI STUDI DI MILANO

CONCORSO PUBBLICO, PER TITOLI ED ESAMI, A N. 1 POSTO DI CATEGORIA D – AREA TECNICA, TECNICO-SCIENTIFICA ED ELABORAZIONE DATI, CON RAPPORTO DI LAVORO SUBORDINATO A TEMPO INDETERMINATO PRESSO L'UNIVERSITÀ DEGLI STUDI MILANO - DIPARTIMENTO DI SCIENZE DELLA SALUTE – CODICE 22201

La Commissione giudicatrice del concorso, nominata con Determina Direttoriale n. 580 del 20.1.2023, modificata con Determina Direttoriale n. 2859 dell'1.3.2023, composta da:

Prof.ssa Elena Menegola	Presidente
Dott.ssa Angela Maria Savino	Componente
Dott. Marinos Kallikourdis	Componente
Sig.ra Francesca Pratico'	Segretaria

comunica i quesiti relativi alla prova orale:

GRUPPO DI QUESITI N. 1

1. Durante un esperimento, uno sperimentatore richiede di migliorare la risoluzione di acquisizione per i campioni rimanenti dopo che è stata acquisita la prima metà dei campioni. Quale sarebbe il comportamento più corretto del tecnico?

2. Sospetto di cross-contaminazione: quale la strategia più corretta?

Brano in inglese:

Types of Confocal Microscopes

Confocal microscopes can be distinguished by their method of scanning. The confocal image is constructed as the illumination point is moved over the sample and several strategies have been developed to accomplish that. In a stage scanning system, like the Minsky configuration, the optics are held fixed and the object is scanned by moving the microscope stage. This method has some advantages including: all points in the image have identical optical properties, edge artifacts are reduced by using only the central axis of the objective lens, and the sample size is limited only by the translation range of the stage itself. However, this requires high mechanical precision for optimal resolution, is slow compared to scanning the beam, and may lead to motion artifacts or rearrangement of tissue due to the force involved in translation. Most modern confocal microscopes scan the illumination beam across the stationary sample and are controlled with an acousto-optic tunable filter (AOTF) to rapidly turn lasers on and off, attenuate the laser power, and select the wavelength during imaging. The major classes of scanning confocal microscopes are described below.

Elliot AD, Confocal Microscopy: Principles and Modern Practices Curr Protoc Cytom. 2020 March ; 92(1): e68. doi:10.1002/cpcy.68.

GRUPPO DI QUESITI N. 2

1. Gestione del campione biologico da parte del tecnico: quali strategie al ricevimento di campioni a fresco?

2. Distacco della corrente elettrica durante la notte: quali procedure?

Brano in inglese:

Sample preparation for high-resolution 3D confocal imaging of mouse skeletal tissue

Embedding and sectioning. In this protocol, we use sucrose along with polyvinylpyrrolidone (PVP) for cryoprotecting26 the bone samples before subjecting them to embedding and freezing. Our experience shows that 20% (wt/vol) sucrose and 2% (wt/vol) PVP solution result in optimal cryoprotection of bones.

Embedding of the bone samples is performed in a gelatin-based solution. Gelatin-based embedding and freezing of sample blocks, which has been used previously for special purposes27, prevents the disintegration of bone fragments, thereby keeping the sections intact during the cutting. Another advantage of gelatin-based embedding is that, if required, gelatin can be removed from the bone sections by soaking them in hot water. The most crucial aspect of gelatin-based embedding is getting the optimal consistency of gelatin. An embedding solution of 8% (wt/vol) gelatin with 20% (wt/vol) sucrose and 2% (wt/vol) PVP has an optimal consistency for complete penetration of bone during the freezing step, which preserves the tissue architecture during sectioning.

Efficient sectioning of bones requires complete freezing of the tissue blocks. Incompletely frozen medium has a sticky consistency and is therefore difficult to section in a cryotome. Cryotome settings, such as speed of sectioning, need to be adjusted to the thickness of sections and bone properties. Slower speeds will improve section quality. Thick sections are easy to cut and handle, but they are more difficult to mount on glass slides, and thin sections fail to provide 3D information on tissue organization and vascular architecture; for the purposes of 3D imaging addressed by this protocol, we recommend 70–150 µm. However, this protocol can be used to generate thinner sections; greater care should be taken while taking thin sections, which requires less cutting speed and greater dexterity.

Anjali P Kusumbe1,2, Saravana K Ramasamy1,2, Andrea Starsichova1,2 & Ralf H Adams1,2

Published online 29 October 2015; doi:10.1038/nprot.2015.125

GRUPPO DI QUESITI N. 3

1. Gestione delle linee cellulari da parte del tecnico: quali strategie per linee in sospensione o ad adesione?

2. Il Dipartimento decide di cambiare il fornitore di Fetal Calf Serum: quale il conseguente comportamento corretto?

Brano in inglese:

High-resolution 3D imaging uncovers organ-specific vascular control of tissue aging

Immunostaining
For immunostaining analyses, tissue sections were air-dried for 15 min and hydrated with PBS. After permeabilization in 0.3% Triton X-100 for 10 min and blocking in 5% donkey serum at room temperature (RT), samples were incubated with primary antibodies diluted in blocking buffer (1:150) overnight at 4ÅãC or for 4.5 hours at RT. Following seven washes (each for 3 min) in PBS solution, sections were then incubated with Alexa Fluor–conjugated antibodies (1:300) for 1.5 hours at RT, washed five times further, and flat-mounted on microscope glass slides with Fluoromount-G (Invitrogen, 00-4958-02). Nuclei were counterstained with TO-PRO-3 or 4′,6-diamidino-2-phenylindole (DAPI). For negative controls, immunostaining with no primary antibodies was performed. Primary antibodies used are provided in table S1. Secondary antibodies used are as follows: donkey anti-rat immunoglobulin G (IgG) Alexa Fluor 594 (A-21209, Thermo Fisher Scientific), donkey anti-goat IgG Alexa Fluor 488 (A-11055, Thermo Fisher Scientific), donkey anti-goat IgG Alexa Fluor 647 (A-21447, Thermo Fisher Scientific), donkey anti-goat IgG Alexa Fluor 546 (A-11056, Thermo Fisher Scientific), Alexa Fluor 488 streptavidin conjugate (S11223, Thermo Fisher Scientific), Alexa Fluor 546 streptavidin conjugate (S11225, Thermo Fisher Scientific), donkey anti-rabbit IgG Alexa Fluor 488 (A-21206, Thermo Fisher Scientific), and donkey anti-rabbit IgG Alexa Fluor 647 (A-31573, Thermo Fisher Scientific).

Junyu Chen1,2*, Unnikrishnan Sivan1*, Sin Lih Tan1*, Luciana Lippo1*, Jessica De Angelis1*, Rossella Labella1*, Amit Singh1,3, Alexandros Chatzis1, Stanley Cheuk4,5, Mino Medhghalchi6, Jesus Gil7,8, Georg Hollander4, Brian D. Marsden6,9, Richard Williams6, Saravana K. Ramasamy7,8, Anjali P. Kusumbe1†

Milano, 24 maggio 2023

La Commissione

Prof.ssa Elena Menegola - Presidente .....

Dott.ssa Angela Maria Savino - Componente .....

Dott. Marinos Kallikourdis - Componente .....

Sig.ra Francesca Pratico' - Segretaria .....