UNIVERSITÀ DEGLI STUDI DI TORINO

Cell Adhesion Assay performed on Tecom Dental Implants

Customer: Titanmed s.r.l.

Via Nizza, n. 230–10127 Torino – Tel. 011.6708360 – e-mail: federico.mussano@unito.it

Aim of the study:

This work aimed at studying the morphology of cells grown at the interface with titanium dental implants manufactured by Titanmed s.r.l.

The physico-chemical properties of the material surface are essential for predicting the behavior of dental implants placed within the jaws. The surface topography, in terms of roughness and porosity, influences cell adhesion, proliferation and differentiation: cell structural details and cell morphology are the response to stimuli from the cell-surface contact.

Based on these considerations, we evaluated the adhesion of murine osteoblast precursors (MC3T3), which represent the best model in this field of application, widely supported by scientific literature. The use of pre-osteoblasts, indeed, is increasingly preferred to the cell lines obtained from osteosarcomas (eg. SaOS-2, MG-63, etc.) as they are neoplastic and therefore might not be the best tool for mimicking in vitro –although in a very simplified setup- the normal bone healing.

Moreover, also the primary cultures, namely cell clones isolated from tissues of donors, whenever appropriate, are hindered by a great individual variability, which does not allow a satisfactory experimental reproducibility.

Materials:

Tests were conducted on the implant provided by Titanmed srl, which was received perfectly packaged and sterile. In particular, as reported in the delivery note (n.2363, 29th September 2014), the following sample was analyzed:

- Implant lot 002520 with surface treatment SL TECOM IMPLANTOLOGY

Methods:

The cells used for the adhesion tests are precursors of murine osteoblasts (MC3T3-E1), which were purchased from the "European Collection of Cell Cultures." All the cell adhesion assays were conducted in accordance with the protocols available in the international scientific literature.

MC3T3-E1 cells were plated at a density of 150,000 cells/well on a 12-well multiwell (12-well, Corning) containing the implant supplied as a sample (always manipulated in sterile conditions). The cells were then cultured in MEMα, supplemented with 10% Fetal Bovine Serum (Sigma-Aldrich Co. LLC.), 2mM Glutamine, 1% penicillin–streptomycin (MD Biomedicals, Thermo Fisher Scientific, Waltham, MA) under a humidified atmosphere of 5% CO_2 in air at 37 ° C.

The experimental handling was completely performed under sterile vertical laminar flow hood within biosafety cabinets, certified level 2. One day (24 h) after plating, the implant was harvested, washed in Dulbecco's Phosphate Buffered Saline (DPBS, Gibco, INVITROGEN Srl) and fixed in 4% paraformaldehyde solution in DPBS. After 3 washes in DPBS, cells were permeabilized in a solution of 0.1% Triton X100 (Sigma-Aldrich Co. LLC.) in DPBS. The sample was then washed 3 times in DPBS.
Cells were labeled with Rhodamine phalloidin (Life Technologies) in order to mark the cytoskeleton (red fluorescence). The implant was subsequently washed 3 times in DPBS. The cell nuclei were labeled using DAPI (Life Technologies) (blue fluorescence). The implant was finally washed 3 times in DPBS.
The sample was analyzed to a fluorescence microscope Nikon Eclipse Ti-E where images of adherent cells on the implant were acquired. To properly depict the cell adhesion on the surfaces observed the images were processed so as to combine the different fluorescence channels and make appreciable the morphological details considered.

Results:

The choice of marking and analyze actin (a fundamental component of the cell cytoskeleton) was successful. The cytoskeleton is the scaffold of cells and is responsible for the movement and adhesion on surfaces.

This permitted to assess with great sensitivity the cell morphology as well as the arrangement of filopodia and lamellipodia, thus leading to thoroughly appreciate the cell spreading. These factors appear to be good criteria to evaluate cellular "comfort" on surfaces and measure how these surfaces are adequate to support a proper cell growth and development.

The surface of the analyzed implant is, from a biological perspective, optimal to allow a good osseointegration. Indeed, it is possible to appreciate (by the representative images and through the assessment made by the operator in the analysis) the uniformity of cell growth and adhesion of the entire implant (i.e., excluding the presence of zones and areas with quality defects). At higher magnification, it is even possible to distinguish the details of the cellular microstructure and appreciate how cells grow in a highly branched and dendritic filopodia with long and complex morphology, suggesting an optimal cell growth and adhesion.

Furthermore, by marking nuclei, it is possible to note that 24 hours after plating the cells are already growing and multiplying in number.

Conclusion:

The results highlight the high quality of the implant surface topography and chemistry that allowed adhesion, development and optimal growth of the cells tested within 24 hours after

plating. With regard to these tests, we may conclude that the analyzed implant is adequate to allow a good osseointegration.

Turin, 3rd October 2014.

Sincerely,

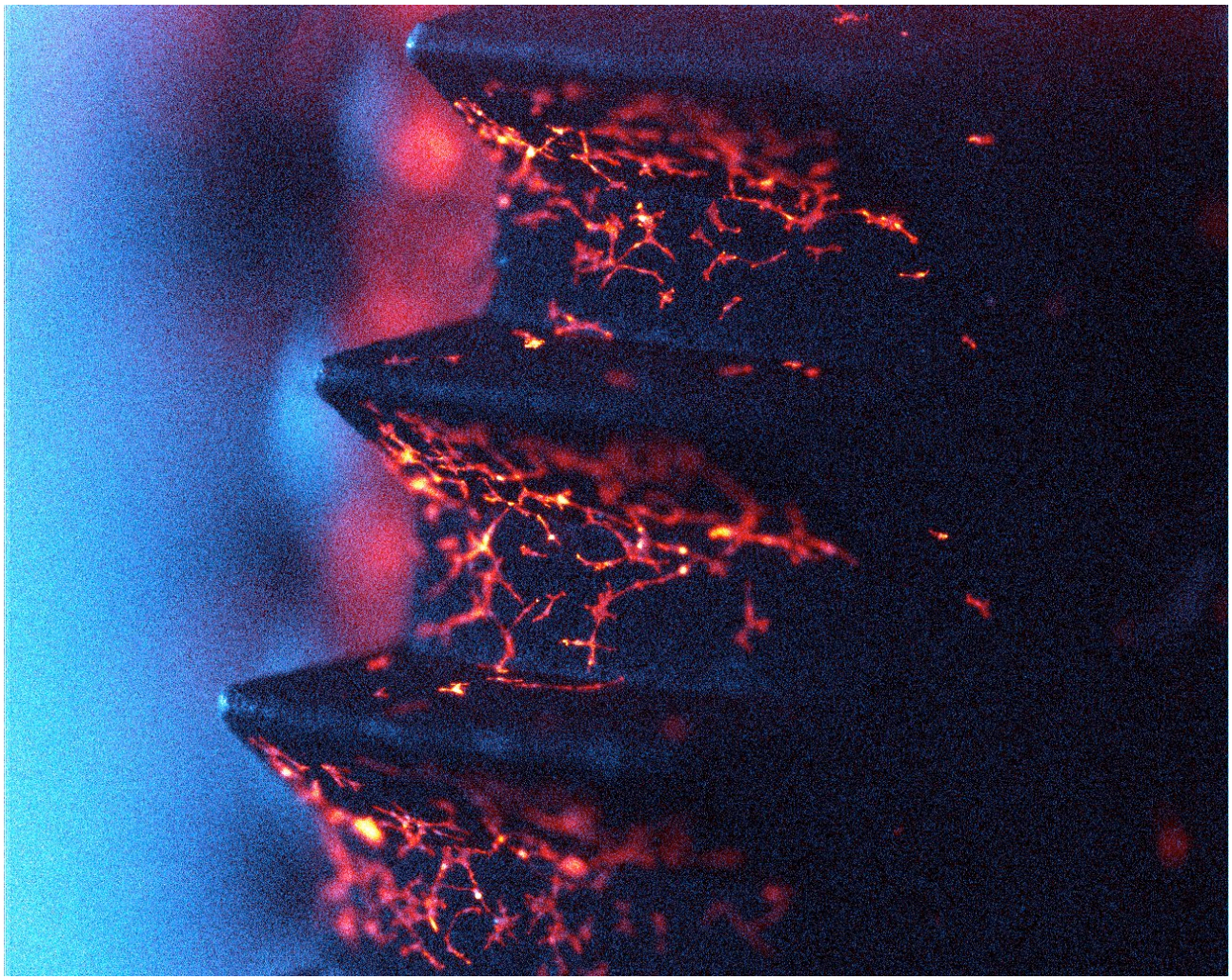
Federico Mussano

Via Nizza, n. 230–10127 Torino – Tel. 011.6708360 – e-mail: federico.mussano@unito.it

UNIVERSITÀ DEGLI STUDI DI TORINO

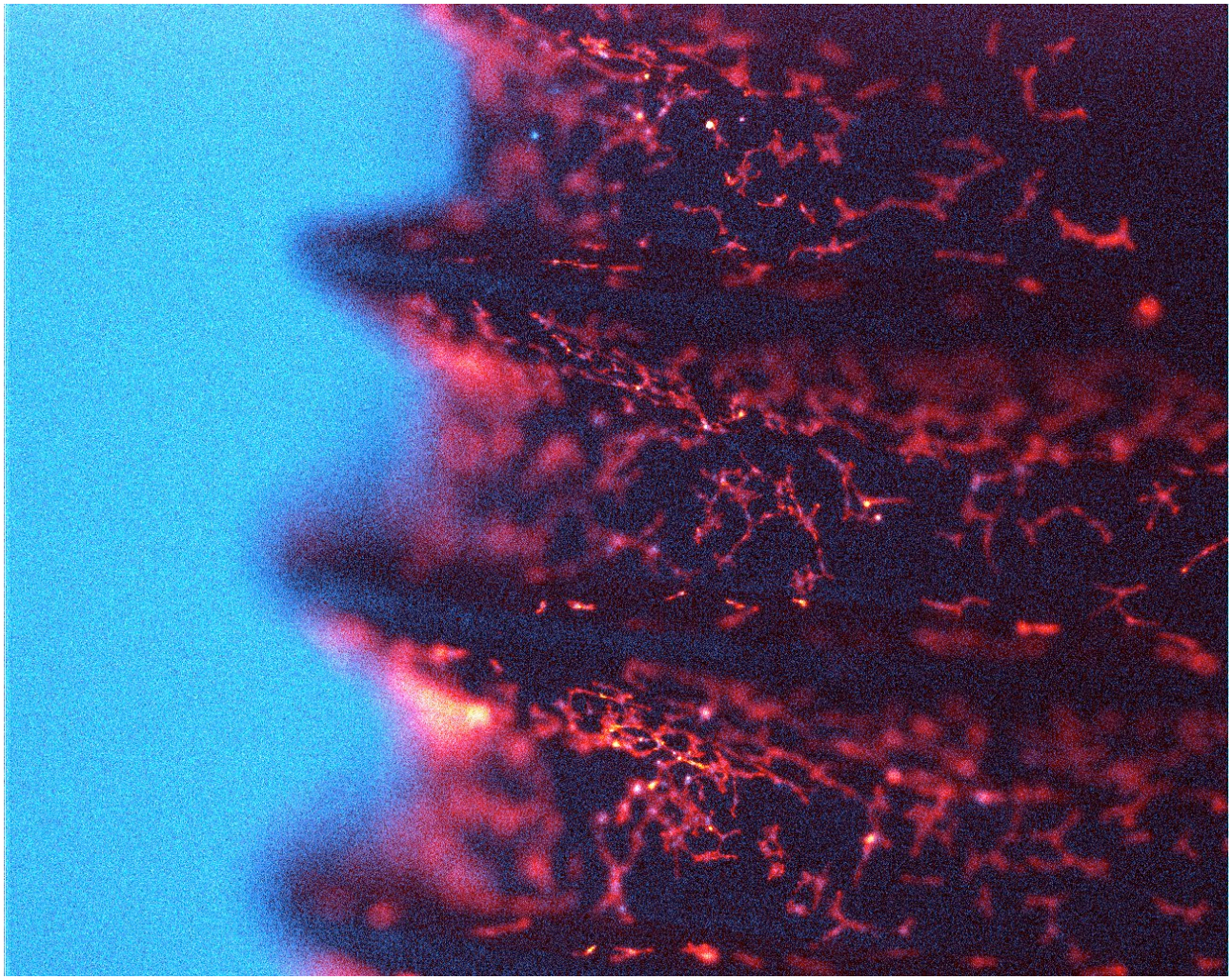
Figures:

Implant lot 002520 with surface treatment SL TECOM IMPLANTOLOGY



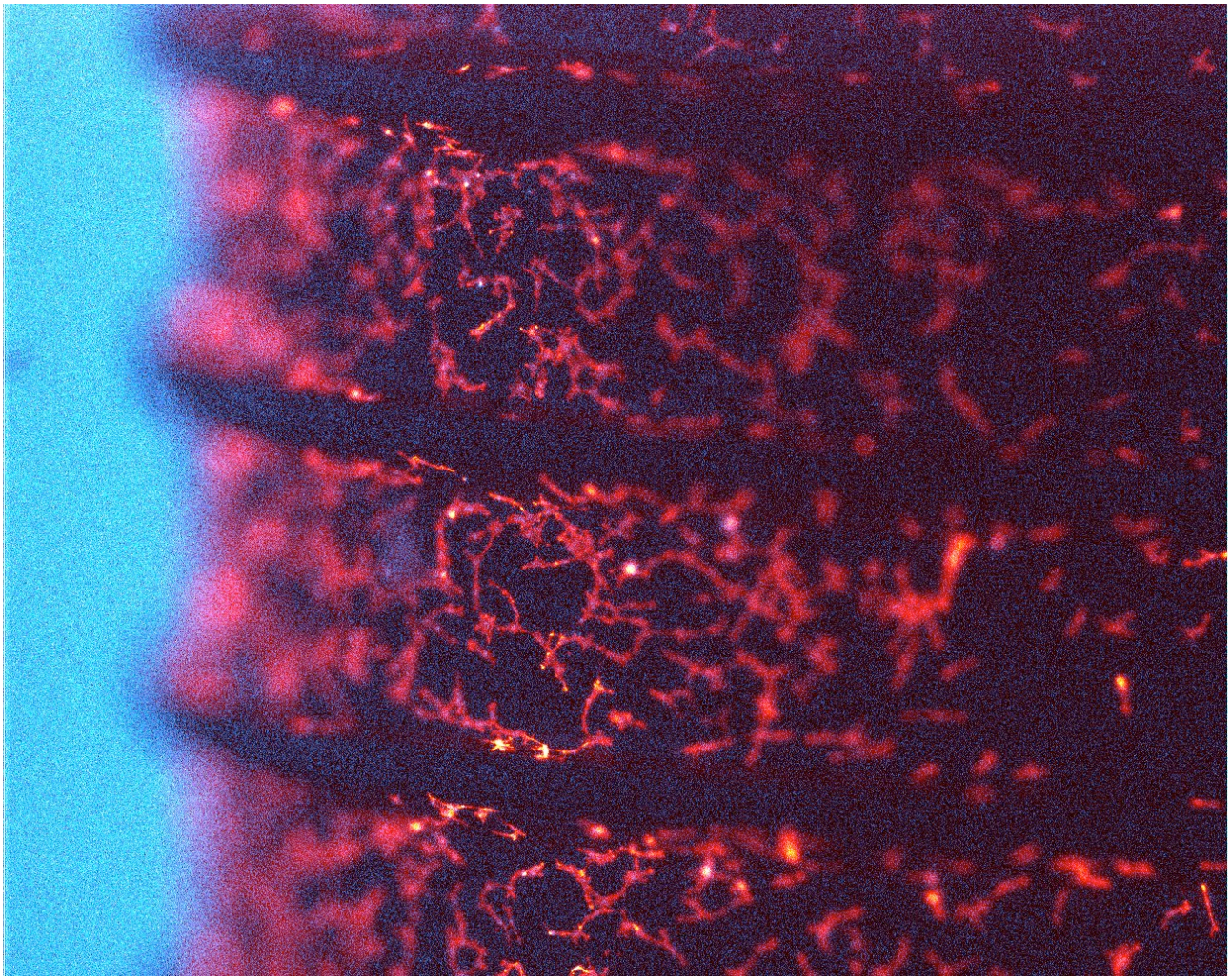
Via Nizza, n. 230–10127 Torino – Tel. 011.6708360 – e-mail: federico.mussano@unito.it

UNIVERSITÀ DEGLI STUDI DI TORINO



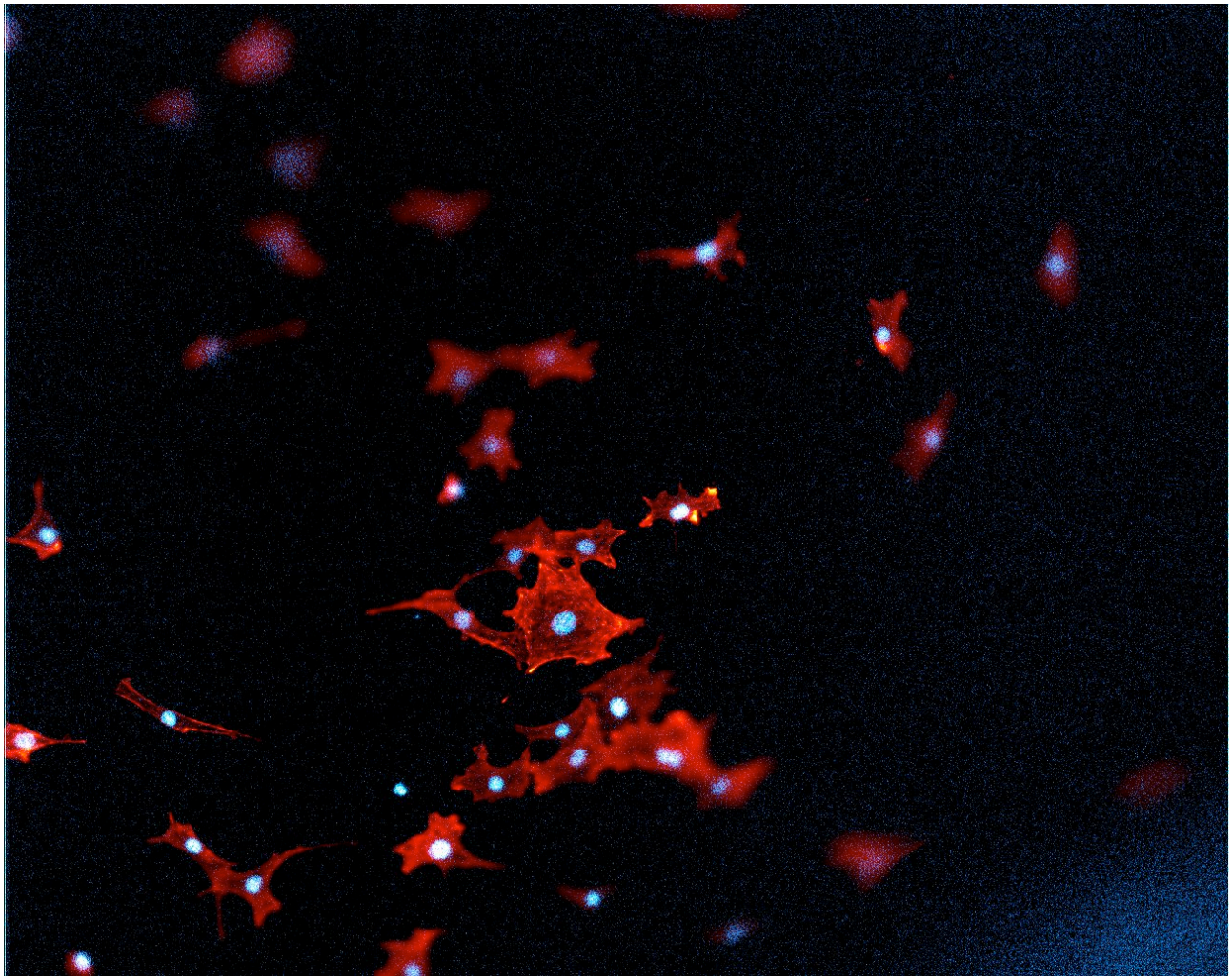
Via Nizza, n. 230–10127 Torino – Tel. 011.6708360 – e-mail: federico.mussano@unito.it

UNIVERSITÀ DEGLI STUDI DI TORINO



Via Nizza, n. 230–10127 Torino – Tel. 011.6708360 – e-mail: federico.mussano@unito.it

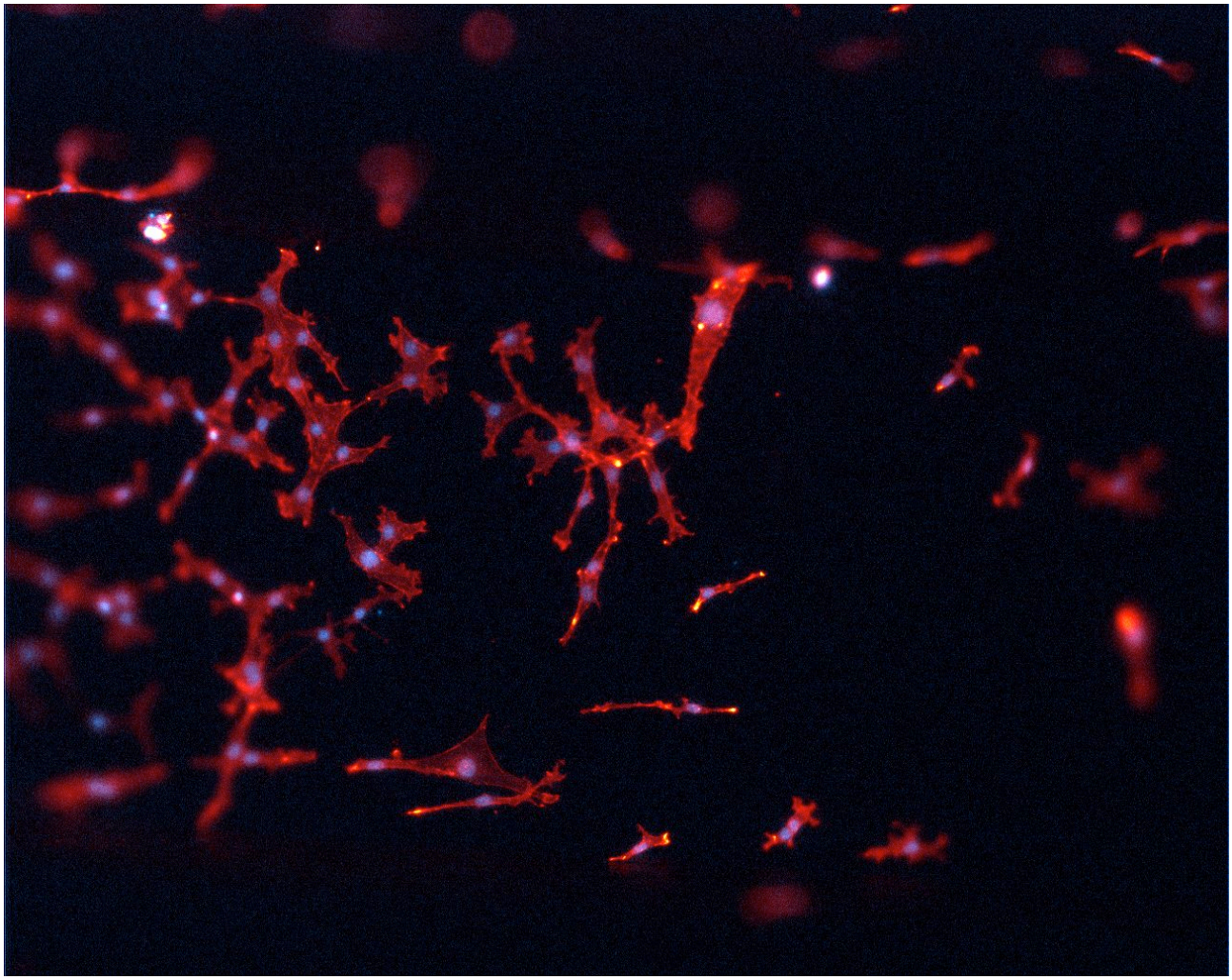
UNIVERSITÀ DEGLI STUDI DI TORINO



Details at higher magnification

Via Nizza, n. 230–10127 Torino – Tel. 011.6708360 – e-mail: federico.mussano@unito.it

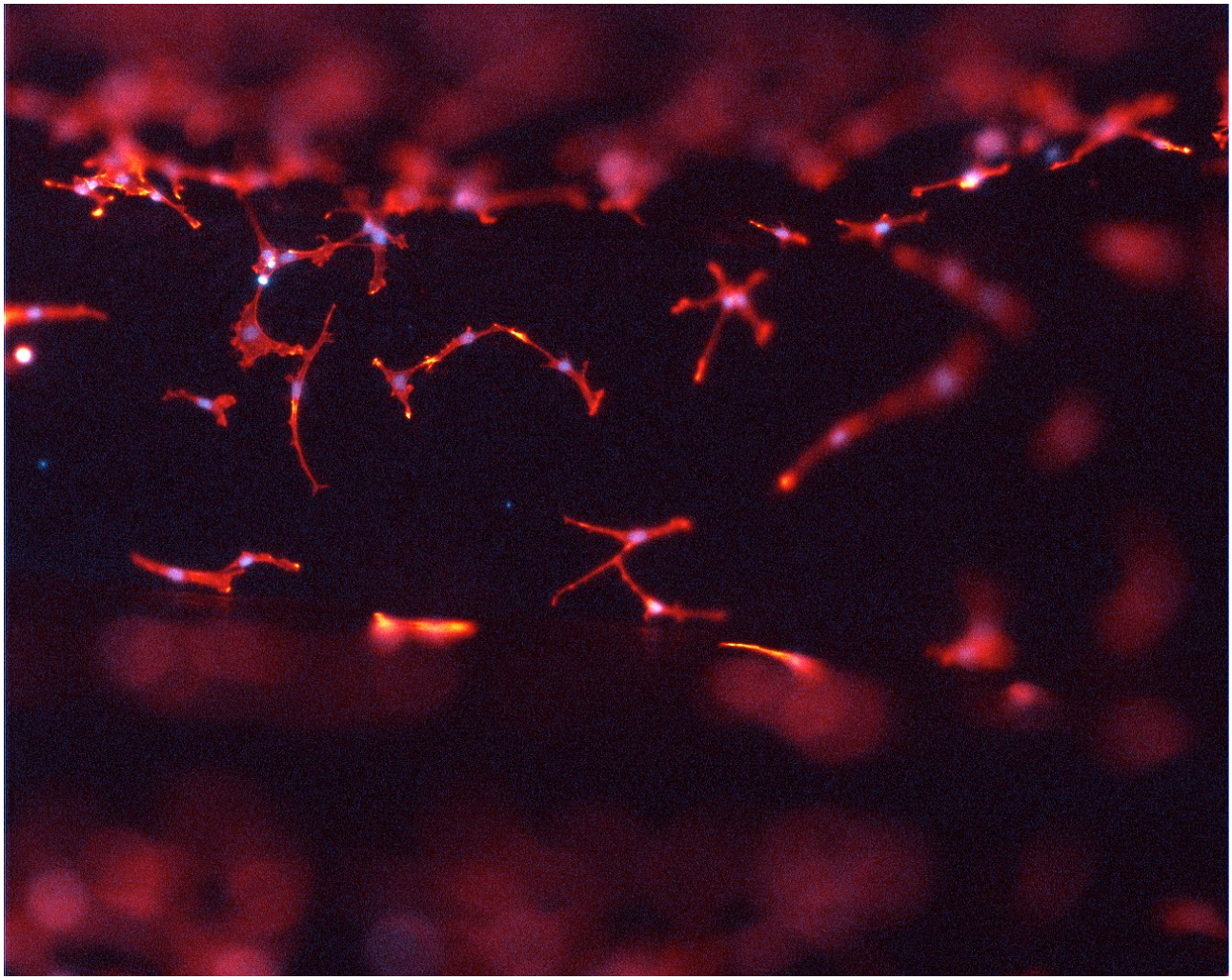
UNIVERSITÀ DEGLI STUDI DI TORINO



Details at higher magnification

Via Nizza, n. 230–10127 Torino – Tel. 011.6708360 – e-mail: federico.mussano@unito.it

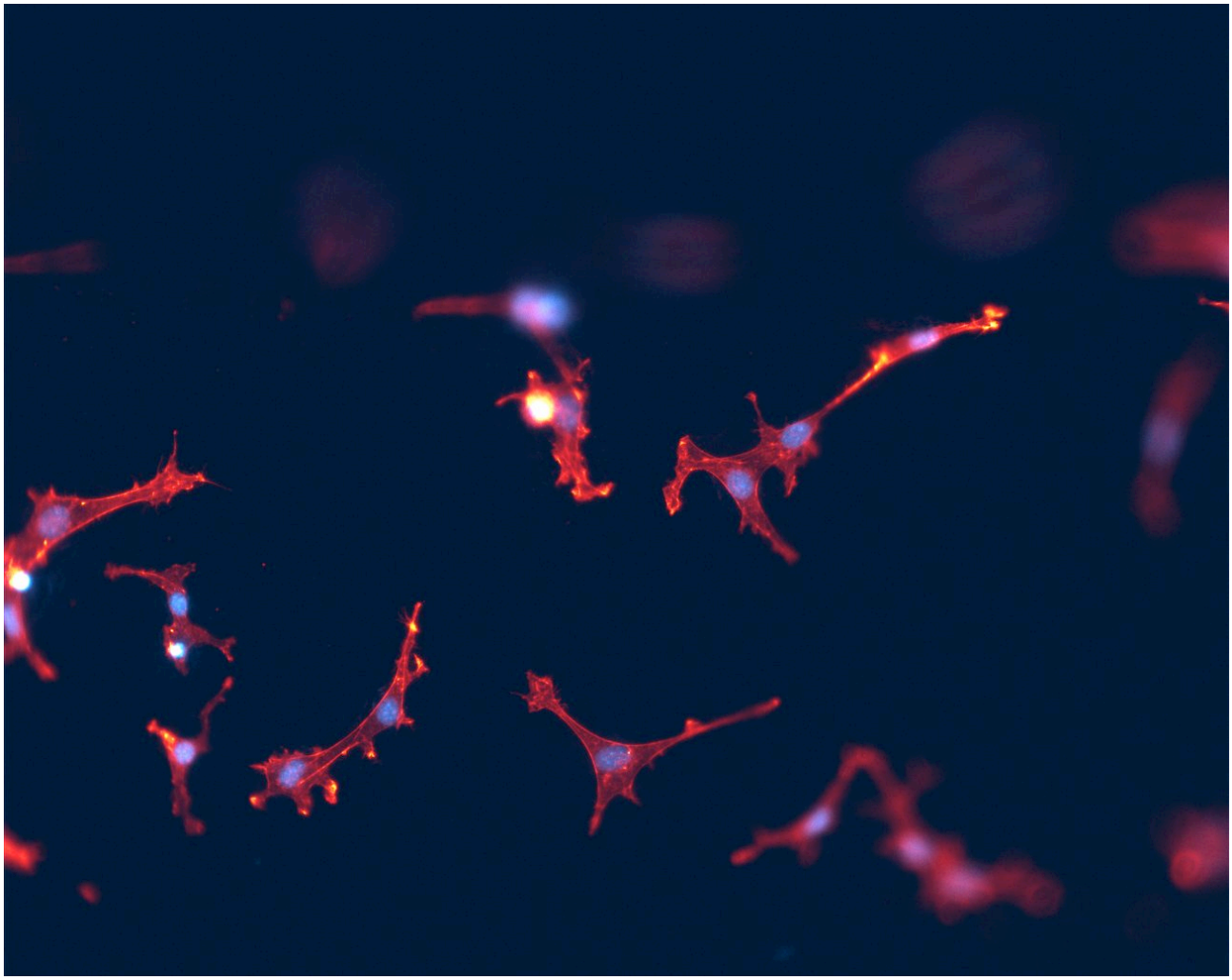
UNIVERSITÀ DEGLI STUDI DI TORINO



Details at higher magnification

Via Nizza, n. 230–10127 Torino – Tel. 011.6708360 – e-mail: federico.mussano@unito.it

UNIVERSITÀ DEGLI STUDI DI TORINO



Details at higher magnification

Via Nizza, n. 230–10127 Torino – Tel. 011.6708360 – e-mail: federico.mussano@unito.it