The **Nanomedicine team in the Department of Chemical and Biological Engineering** has been using the UNM Cancer Center Fluorescence Microscopy Shared Resource to capture fluorescent confocal images of nanoparticle trafficking and drug delivery within antigen presenting dendritic and cancer cells. Projects include the development of nanoparticle vaccines; multi-modal imaging of therapeutic responses to combination nanoimmunotherapy and checkpoint inhibitor blockade; and trafficking of drug-loaded lipid encapsulated mesoporous silica nanoparticles. Microscopists include Dr. Rita Serda, Research Assistant Professor; UNM Engineering STEP undergraduate students Kimberly Denman and Angelea Maestas; and NMSU REU undergraduate student Karen Sanchez. Research is conducted in the C. Jeffrey Brinker Laboratory in Centennial Engineering with support from both a UNM HSC Cancer Center Pilot Project Award (Selwyn, Serda, Norenberg and Brinker), and LDRD and AFOSR funding from Sandia National Laboratories (Brinker).

**Figure 1. Nanoparticles for immunotherapy.** This micrograph supports robust internalization of nanoparticle-based vaccines by dendritic cells. Actin: red, rhodamine phalloidin; Microtubules: green, Alexa Fluor 488 anti-tubulin antibody; nuclei: blue, DAPI; antigen and adjuvant loaded mesoporous silica nanoparticles supported with a lipid bilayer; white; DyLight 633. The image was acquired using the Leica TCS-SP8 Confocal Microscope. **Credit: Angelea Maestas**

**Figure 2. Drug delivery.** Three-dimensional iso-surface-rendered images of an A549 lung cancer cell one hour after introduction of lipid encapsulated mesoporous silica nanoparticles (red; DyLight 633) loaded with CRISPR RNP (green, Alexa Fluor 488). The image was acquired using the Leica TCS-SP8 Confocal Microscope and the threshold for the actin label (Phalloidin Rhodamine) was incrementally increased to show first the cell cytoskeleton and last the perinuclear localized nanoparticles. **Credit: Dr. Rita Serda**

**Figure 3. Nanoparticles in orbit around the nucleus.** Three-dimensional iso-surface rendered image of a cell nucleus (blue; DAPI) with lipid (red) encapsulated mesoporous silica (cyan) nanoparticles in orbit. The image was acquired using the Leica TCS-SP8 Confocal Microscope equipped with a 63x oil objective. **Credit: Dr. Rita Serda**

**Figure 4. Protocell internalization by HeLa cells.** The micrograph shows uptake of protocells (mesoporous silica nanoparticles coated in lipids) by HeLa cells. Nuclei are stained with DAPI (blue), actin is stained with phalloidin (green) and protocells are labeled with Cy3 (red). The image was obtained using the Zeiss LSM 800 Airyscan Confocal Microscope equipped with a 63x oil objective. **Credit: Karen Sanchez**

**Top row:** Kimberly Denman (left) and Karen Sanchez (right); **Bottom row:** Rita Serda (left) and Angelea Maestas (right).
Whole mount mouse retinal vasculature immunostained with Cy3 anti-Armenian hamster CD31 were imaged on the Leica SP8 confocal microscope using the tiling feature and resonance scanner. A sub-stack of optical sections for each vessel layer (A) microvasculature or capillary bed; (B) pre-capillary arterioles/venules; (C) Intra-retinal arteries/veins; and (D) composite image was created from the merged tiles. Maximal projection of each sub-stack shows a different blood vessel pattern in each layer. Credit: Dr. Virginia Yao
Figure A: Cover Cancer Research
Human prostate cancer selectively metastasizes to the bone with mechanisms that are partially unknown. RAGE is a receptor overexpressed by prostate cancer cells whose expression correlates with the capacity of colonizing the bone marrow microenvironment. Protease 3 (PR3) is a serine protease produced and released by myeloid cells, and has been identified as a RAGE-interacting protein. Using high resolution confocal microscopy (Pinhole 0.6), it was found that soluble PR3 (red) accumulates at the surface of prostate cancer cells overexpressing RAGE (green). This result supports further evidence of heterotypic cell-cell interactions between prostate cancer cells expressing RAGE and hematopoietic cells expressing PR3. For more details, see article by Kolonin and colleagues on page 3144.

Figure B: Colocalization of the peptide LGRFYAASG-pen with annexin A2. The peptide motif LGRFYAASG identified by a screening with an internalized phage (iPhage) display library in tumor cells was selected as cytosol targeting peptide. The penetratin-fused version of the selected peptide (LGRFYAASG-pen) co-localizes and specifically accumulated in the cytoplasm at the cell edges and cell-cell contacts (Staquicini et al., Scientific Reports, June 2017). Credit: Dr. Daniela Staquicini

Figure C-D: Intracellular targeting of annexin A2 by LGRFYAASG-pen disassembles focal adhesions, actin filaments and lipid microdomains (Staquicini et al., Scientific Reports, June 2017). Credit: Dr. Daniela Staquicini