

Organelle Mapping of the Human Proteome - Towards a Subcellular Atlas

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Abstract

Information on protein localization on organelle level is important to map and characterize the human proteome and to better understand cellular functions of proteins. Here, we report on the development and progress of a confocal subcellular protein atlas, as part of the Human Protein Atlas (HPA) portal. In this third release, the subcellular data comprises approximately 24000 high-resolution confocal images corresponding to the analysis of almost 4000 proteins in three human cell lines.

Experimental

Three different human cell lines (A-431 epidermoid carcinoma; U-251MG glioblastoma and U-2OS osteosarcoma) were carefully chosen for the localization study. The cells were grown in glass-bottomed 96-well plates, fixed and permeabilized using a formaldehyde/detergent treatment. Antibodies generated within the HPA program (1) were subsequently used for immunofluorescent staining together with organelle markers for nuclei, microtubules and endoplasmic reticulum (see fig. 1). The fixation and staining procedures were automated using a pipetting robot. Two high-resolution confocal images of each antibody/cell line were acquired using a Zeiss 510 Meta laser scanning microscope and the microscope operator ensured that they were representative for the sample. All images were manually annotated in terms of protein localization, staining intensity and characteristics (see fig. 2, 3 and 4).

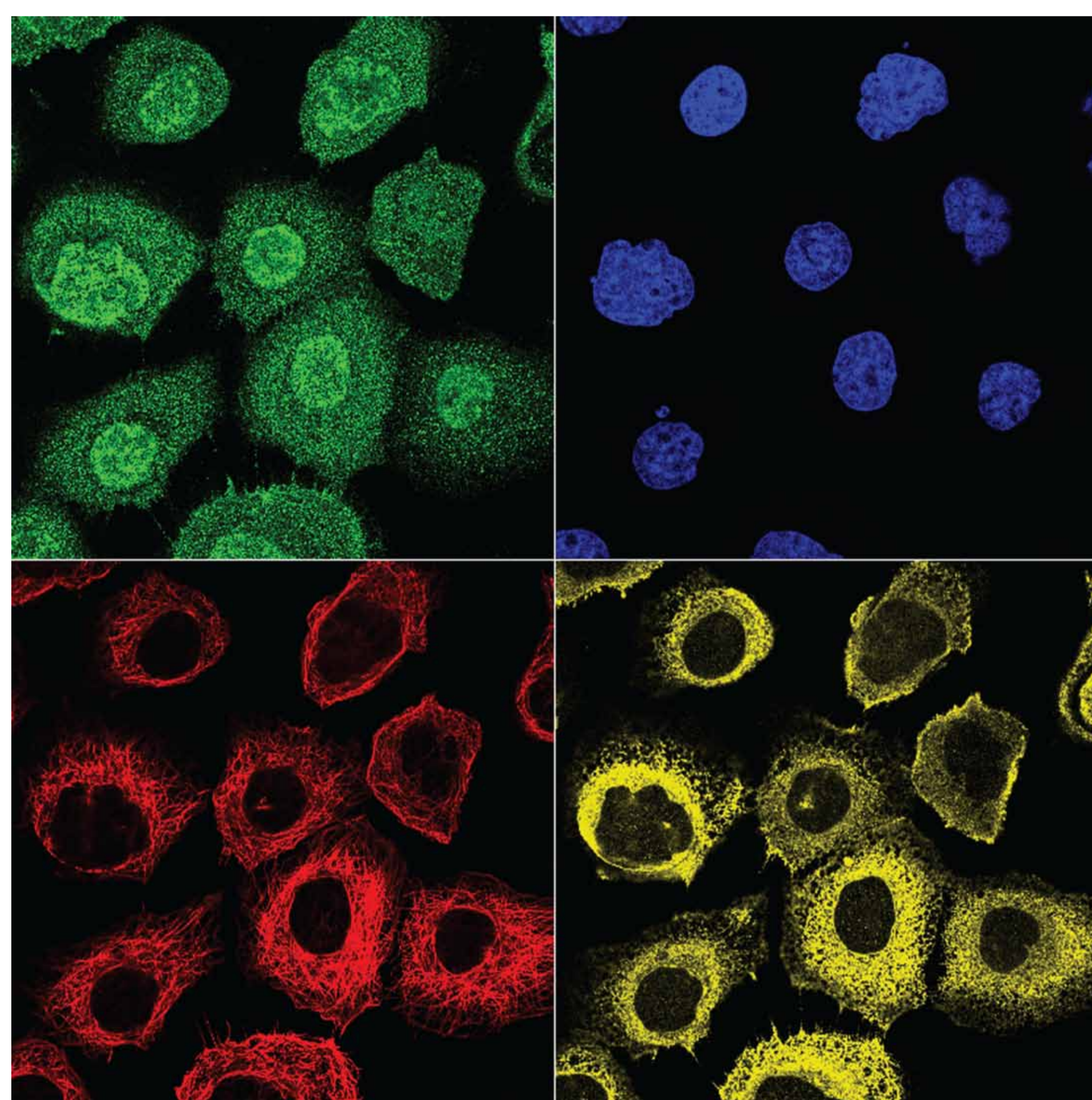


Figure 1. The four immunofluorescent markers used. HPA-antibody specific (green), staining of nucleus with DAPI (blue), cytoskeleton (red), endoplasmic reticulum (yellow).

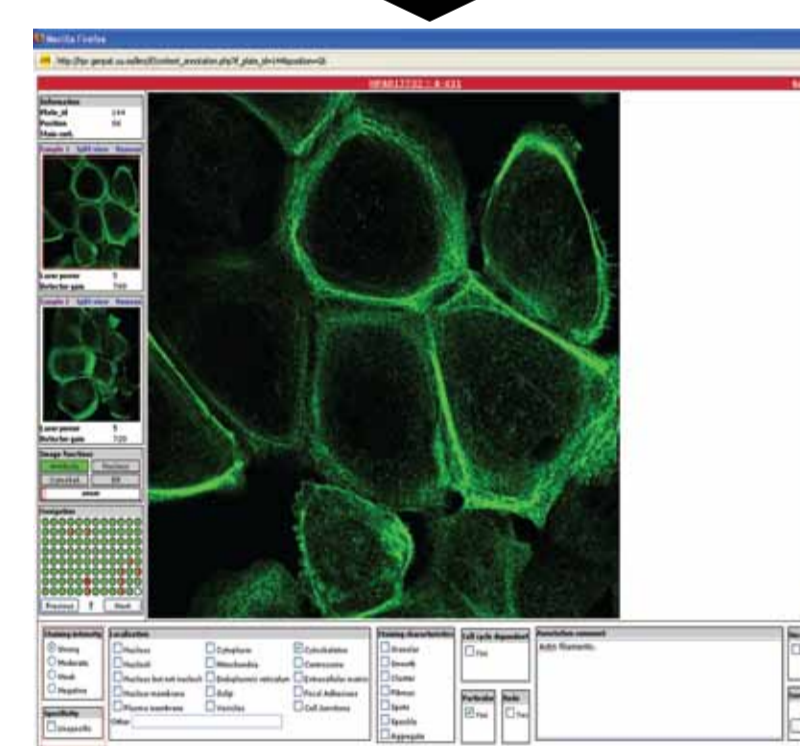
Workflow



Automated fixation and staining by the Matrix Plate-Mate 2x2 pipetting robot



Image acquisition with Zeiss 510 Meta laser scanning microscope



Annotation of subcellular localizations

Figure 4. Workflow of the production.

- References
- (1) M Uhlén et al (2005), "A human protein atlas for normal and cancer tissues based on antibody proteomics", Mol Cell Proteomics 4(12):1920-32.
 - (2) L Barbe et al (2007), "Towards a confocal subcellular atlas of the human proteome", Mol Cell Proteomics 7(3):499-508.
 - (3) Berglund et al (2008) "A gene-centric protein atlas for expression profiles based on antibodies", Mol Cell Proteomics 7: 2019-202

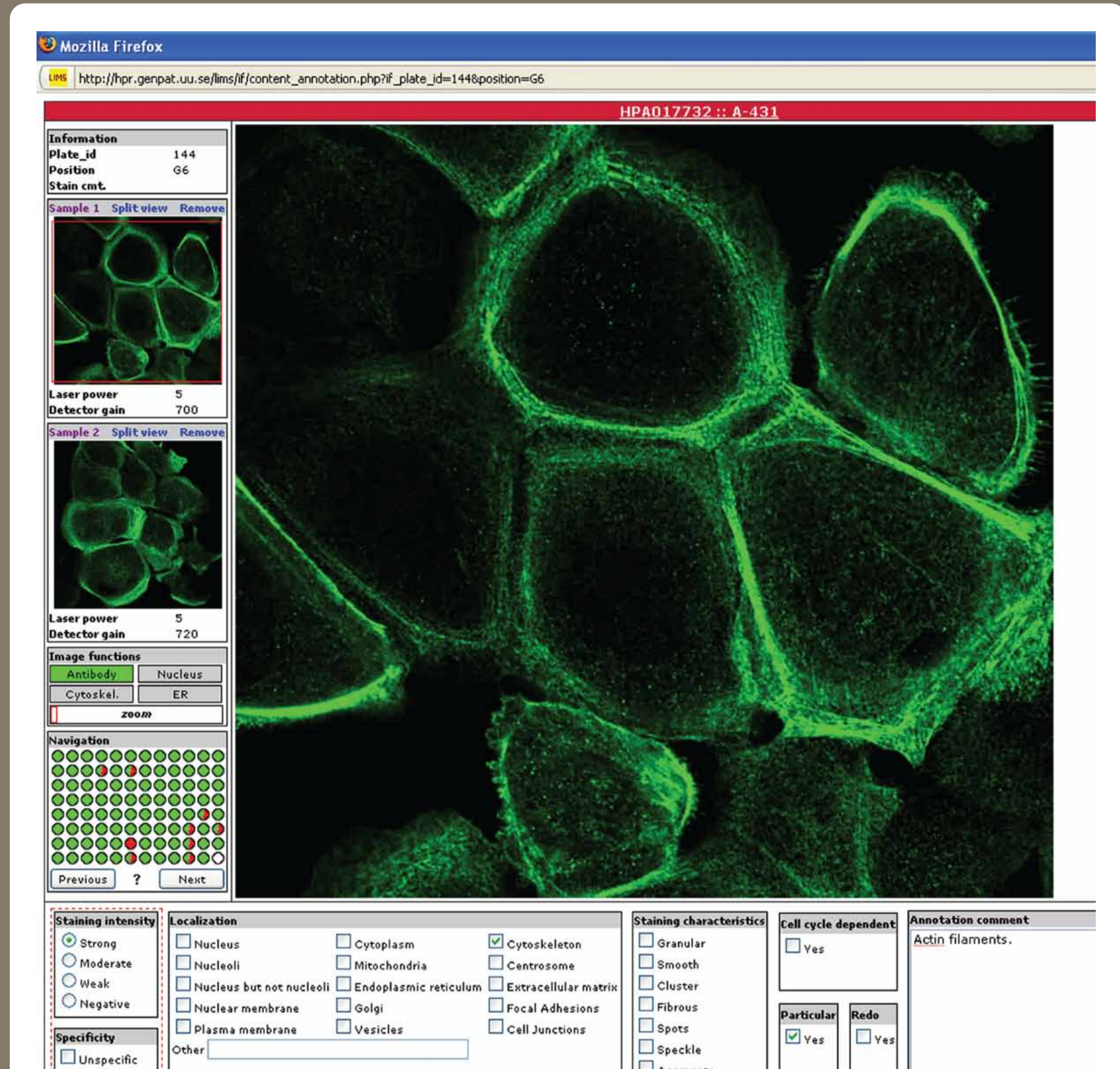


Figure 2. Screen dump of annotation of subcellular localizations. All data management as well as annotation is supported by LIMS platform developed by inhouse IT.

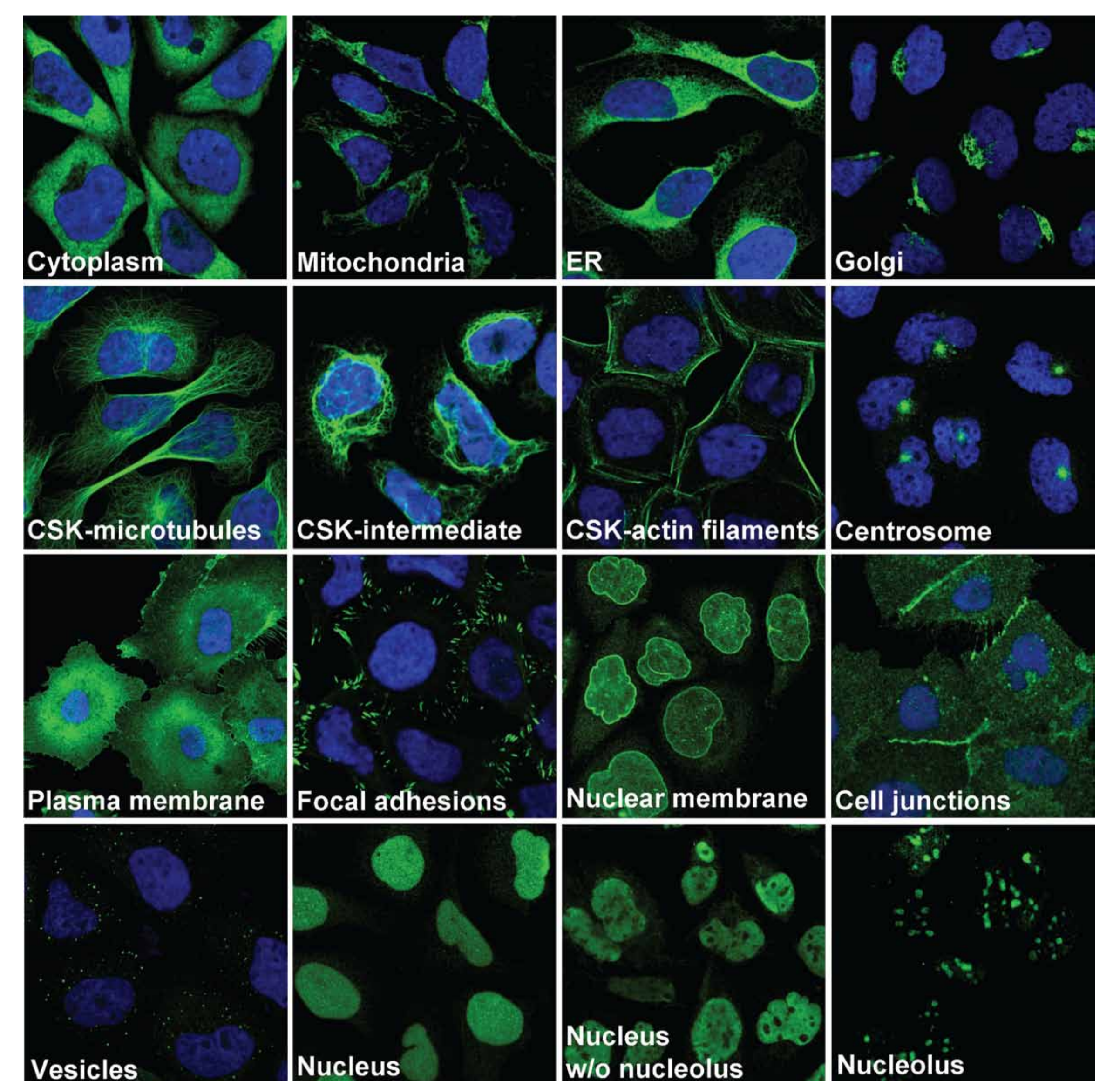


Figure 3. Examples of subcellular localizations that are annotated.

Result and discussion

The images constitute a confocal (2) subcellular atlas, publicly available as part of the HPA portal (www.proteinatlas.org) (3). The third release of this atlas comprises approximately 4000 proteins analyzed in three human cell lines. About 24000 high-resolution confocal images, annotated in terms of subcellular localizations, staining intensity and characteristics, will be available. In total, 17 different localizations were annotated and over 80% of the analyzed proteins could be classified into one or more of these. The obtained subcellular localizations were further assessed in comparison to literature and a reliability score (supportive, uncertain or non-supportive) was provided for all proteins.

Conclusions

This is the first large-scale, antibody-based study to localize proteins into subcellular compartments using confocal microscopy. The atlas aims to provide publicly available information on subcellular information for all human proteins with the goal to facilitate functional studies of proteins and the discovery of novel biomarkers.

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www.proteinatlas.org

