Correlative light microscopy for high-content screening

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BioTechniques 55:243-252 (November 2013)
doi 10.2144/000114099

Keywords: super-resolution microscopy; dSTORM; high-content screening; correlative microscopy; Golgi complex

High-throughput microscopy is an effective tool for rapidly collecting data on a large scale. However, high throughput comes at the cost of low spatial resolution. Here we introduce correlative light microscopy by combining fast automated widefield imaging, confocal microscopy and super-resolution microscopy. We demonstrate the potential of this approach for scalable experiments. The workflow consists of a robust approach for selecting cells of interest on a wide-field screening microscope at low resolution and subsequently re-localizing those cells with micrometer precision for confocal and super-resolution imaging. As a case study, we visualized and quantified cis- and trans-Golgi markers at increasing resolution.

***Method Summary:** Quantitative description of biological systems is the major challenge of biological research. Fluorescence microscopy plays an important role because it allows researchers to not only visualize but also evaluate virtually every cellular process of interest. Fluorescence microscopy, however, comes with a trade-off between throughput and spatial/temporal resolution. Due to these technological limitations, imaging biological samples is generally constrained to either high throughput but low resolution or high resolution but low throughput.*

Introduction

High-throughput widefield or spinning disk confocal screens can process large numbers of targets. For example, RNAi screens can be performed with a sample number sufficient to cover the whole human genome (~23,000 genes). To obtain a reasonable number of cells per field of view and to ensure fast imaging, low to medium spatial resolution is usually chosen (10× air or 40× water objective) (1,2).

Point scanning confocal microscopy is applicable to small-scale experiments and is often used following data collection by high-throughput methods (3). In order to increase the information content of small-scale confocal screens, automated correlative screening techniques (4), which combine low with high-resolution point scanning, may be used for targeting relevant phenotypes and zooming closer into interesting events by imaging in a mode of high information context.

The spatial resolution in both widefield and confocal microscopy is limited by diffraction. In recent years, a new generation of microscopy techniques has been developed that bypass this limit in resolution (5–7). Among these are single-molecule techniques, which were demonstrated to reach near molecular

spatial resolution (8–12). Additionally, co-localization analysis can be refined by using super-resolution data. For example, co-localization analysis at the molecular level was demonstrated, attributing a co-localization value to each single biomolecule detected (13,14).

In order to combine fast low-resolution widefield methods with slow high- or super-resolution microscopy, we have developed a workflow for correlative microscopy that combines the best features of each. By a fast widefield screen of a large sample, representative cells showing defined phenotypes are selected and subsequently imaged in confocal and super-resolution modes. To be able to image in the different microscopy modes, we have chosen fluorophores that can be used in all three imaging platforms. In this study, we use correlative light microscopy to analyze the distribution of proteins residing at different cisternae in the Golgi complex. We demonstrate significantly improved spatial resolution of GalT (trans-Golgi marker) and GM130 (cis-Golgi marker) under diverse treatments when applying correlative microscopy for the same cell.

Materials and methods

Cell preparation

NRK (normal rat kidney, CRL-6509; ATCC, Manassas, VA) cells that had been stably transfected with the Golgi enzyme GalT tagged with CFP (NRK-GalT-CFP) were cultured in DMEM (GIBCO/Invitrogen, CA) containing 5% fetal bovine serum (FBS), 1% non-essential amino acids (GIBCO/Invitrogen), 2 mM glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin. For experiments cells were plated on µ-Slide 8-well slides (Ibidi, Martinsried, Germany) at densities of 15,000 cells/well 24 h before drug treatment. Poly-L-lysine (Sigma-Aldrich, M0) was applied to µ-Slides for 15 min before cell plating.

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Figure 1. Workflow for correlative imaging. A region of interest is selected on the sample substrate (A) and imaged at low magnification (B). Structures of interest are determined and imaged under different optical conditions (B) or at different microscope systems (confocal, dSTORM) (C,D). (F), (G), and (H) show enlarged regions of (B), (C), and (D), as indicated in (B).

Cell treatment

BFA and nocodazole (Calbiochem/Merck Chemicals, Darmstadt, Germany) treatment was performed at concentrations of 5 $\mu\text{g}/\text{mL}$ and 1 $\mu\text{g}/\text{mL}$, respectively, for 20 min. Cycloheximide (0.1 mg/mL ; Sigma-Aldrich) was added to abolish protein synthesis.

Sample preparation and immunofluorescence

Cells were fixed incubating in 3% PFA for 20 min. TetraSpeck microspheres (0.1 μm ; Invitrogen) were applied and incubated for 15 min, and cells were permeabilized by incubation with 0.1% Triton-X-100 for 5 min. GalT-CFP was counter-stained with anti-GFP (6556, polyclonal rabbit; Abcam, Cambridge, UK) and anti-rabbit Alexa Fluor 532 (Invitrogen). GM130 was stained with anti-GM130 (monoclonal mouse; BD Biosciences, Franklin Lakes, NJ) and anti-mouse Alexa Fluor 647 (Invitrogen).

Widefield microscopy

Widefield images were acquired with an Olympus IX81 microscope with a 10 $\times$ objective lens (Olympus UPlanSApo, NA 0.4; Olympus, Tokyo, Japan) and a field of view of 866 $\mu\text{m} \times 660 \mu\text{m}$. The sample was illuminated by an 150 W Hg/Xe mixed gas arc burner together with appropriate filter combinations for DAPI, Alexa Fluor 532, and Alexa Fluor 647, respectively. Integration times were set to 200 ms (20 ms for DAPI staining), which were the best match to the dynamic range of the CCD camera. In total, 50 fields of view were recorded for each cell selection.

Confocal microscopy

Selected cells were imaged on a Leica TCS SP5 with a 63 $\times$ objective lens (Leica HCX PL APO, NA 1.40; Leica, Wetzlar, Germany), a scan speed of 400

Hz at 512 $\times$ 512 pixels and with a pixel size of 69 nm, resulting in a field of view of 49 $\mu\text{m} \times 49 \mu\text{m}$ (pinhole diameter of 96 μm). For each cell, 47 image planes with an axial distance of 0.2 μm were acquired. Selected cells were initially positioned in the center of the z-stack by an autofocus routine before acquisition. Image acquisition took 196 s at each position, subsequent positions could be acquired every 212 s including positioning of the stage and autofocus routines. In total, confocal multicolor 3-D stacks were imaged at 796 single positions within 46.9 h.

Super-resolution imaging

Super-resolution imaging of selected cells was performed with a custom-built microscope using experimental protocols as described earlier, following direct stochastic optical reconstruction

microscopy (*d*STORM) (11). The beam of a multi-line argon-krypton laser (Innova70C, Coherent, Santa Clara, CA) is coupled into an acousto-optical tunable filter (27896; 8-channel) (AAOptics, Orsay, France) for wavelength selection and then combined with a UV laser beam (405 nm diode, Cube 405–100C, 100 mW, Coherent). The combined beams are focused onto the back-focal plane of an oil-immersion total internal reflection fluorescence objective (PlanApo N 60×, NA 1.45, Olympus) mounted onto an inverted microscope (IX71; Olympus). Emission light is separated from excitation light by appropriate dichroic mirrors and filters (AHF, Tübingen, Germany) and focused on an electron multiplying charged coupled device (EMCCD) camera (Ixon, Andor, Ireland). The samples were mounted on the stage, and the sample chamber was filled with ~200 μ L switching buffer (100 mM β -mercaptoethylamine; MEA in PBS, pH 8). The exposure time was 50 ms with an electron multiplier gain of 200. Imaging intensities were 35 mW at 647 nm excitation wavelength and 65 mW at 532 nm, respectively. To adjust the photoswitching rates of Alexa Fluor 647, illumination with UV light was gradually increased from zero intensity to a maximum intensity of 0.1 mW. The two channels were recorded sequentially (first Alexa Fluor 647, followed by Alexa Fluor 532), chromatic shifts were corrected by using multi-color fluorescent beads (Tetraspeck, Invitrogen). *d*STORM images with were typically reconstructed from 7000 frames using rapidSTORM (15).

Relocation of structures of interest

Relocation was done via coordinate transformation. In order to obtain a coordinate system on the sample that is independent of the coordinate system for the specific microscope system, we fixed three reference structures on the sample. These small pieces of titan coated glass, which each had a circular hole in the titan coating of 400 μ m in diameter, were imaged first in transmission mode. Their central

positions (x and y) were determined by applying an automatic Otsu threshold and calculating the centroid of the resulting area. The z position was taken from the position of the z-stage of the microscope after an autofocus routine. Based on these reference positions, an orthonormal basis of the sample was created, with the first reference position in the top left corner as origin. Widefield images of the sample were acquired, and the position of the lateral microscope stage and the axial z-drive was again recorded for each image. Individual cells of interest were selected for further imaging. The absolute position of these cells (position of the stage + position within the image) was transferred to the microscope stage independent coordinate system of the sample defined by the reference points. To transfer these coordinates to a second system, the referencing procedure was repeated and a coordinate transformation was performed.

Cell selection

Cellular positions for further confocal imaging were selected within the widefield images in two ways: 196 cells were selected manually by an expert marking the cellular positions within the image on a screen, and 600 cells were identified automatically based on nucleus segmentation within the images of the DAPI channel. The confocal images were then investigated for candidates for *d*STORM acquisition and the appropriate cells were chosen manually.

Correlative workflow

Correlative image acquisition was performed as listed here:

1. Image reference markers on widefield setup
2. Image sample automatically on widefield setup
3. Identify target cells for confocal imaging manually / automatically
4. Image reference markers on confocal setup
5. Perform coordinate transformation of cell positions
6. Load position list and image cells automatically on confocal setup
7. Identify targets for *d*STORM acquisition
8. Image reference markers on *d*STORM setup
9. Perform coordinate transformation of cell positions
10. Load position list and image cells on *d*STORM setup

Results and discussion

To achieve sequential correlative imaging on the same sample in three microscopy modes without additional sample preparation steps in between, we first tested various fluorophores for their suitability. Here, we demanded both high photostability and, in particular, the possibility of inducing photoswitching such that single-molecule super-resolution imaging, following the *d*STORM protocol (11), was possible. We identified Alexa

Table 1. Experimental settings and parameters for different microscopy techniques.

Microscope parameters	Widefield	Confocal	<i>d</i> STORM
Objective	10× air, NA 0.4	63× oil, NA 1.4	60× oil, NA 1.4
Field of view	866 μ m × 660 μ m	49 μ m × 49 μ m	55 μ m × 55 μ m
Pixel size	645 nm	69 nm	107 nm
Imaging time (single plane)	~ 0.2 s	~ 1.28 s	~ 300 s
Duration of autofocus	~ 2 s	~ 5 s	N/A
Estimated irradiation density	<10 W/cm ²	<1 kW/cm ²	One – 5 kW/cm ²

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Fluor 532 and Alexa Fluor 647 as an optimal pair for dual-color microscopy. Both fluorophores showed excellent photostability and were thus well suited for widefield and confocal imaging, as well as super-resolution imaging.

We set up a workflow as described in Materials and Methods to target structures of interest within low-resolution wide-field images, determine their spatial coordinates and relocate their positions on multiple other microscope systems. This workflow was applied here in the following way: After widefield imaging, we first transferred the sample to a confocal setup and then to a single-molecule localization microscope and imaged in these systems the same cells of interest identified in the widefield images. In widefield images at 10 $\times$ magnification,

cells of interest that showed the expected phenotype were selected and their positions (coordinates) were determined. The sample was then transferred to a confocal microscope. Using the coordinates, a confocal 3-D stack was acquired for each cell. The precision at which cells of interest could be re-localized was in the range of 10 μ m, which was sufficient to position the whole cell of interest within the field of view of $\sim$ 50 μ m. The sample was then passed on to the single-molecule localization microscope where selected cells of interest based on the confocal images were acquired. The re-localization of cells of interest here was slightly worse, since the reference structures could not be fitted within the field of view, and their center positions had to be estimated. However and more importantly, the cells of

interest could easily be identified. Images acquired during this workflow are depicted in Figure 1 and illustrate the increase of resolution and information content achieved by this correlative approach. Imaging parameters for the different microscope techniques used are given in Table 1.

Co-localization analysis in confocal and high-resolution imaging

We then analyzed the capability of our correlative microscopy approach to compare the distribution of the *cis*- and *trans*-Golgi markers in NRK cells under diverse conditions (16,17). Therefore, we imaged the cell samples using the workflow described above.

Our analysis proceeded as followed: Figure 2 shows the increased resolution

Figure 2. Evaluation of the spatial distribution of GM130 and GalT by confocal and dSTORM imaging. NRK-GalT-CFP cells were treated with nocodazole for 20 min to induce fragmentation of the Golgi complex. Cells were fixed and stained for the endogenous GM130 (see Materials and methods). The cell with the fragmented Golgi complex imaged in a confocal mode is shown in (A). Red represents GM130 and color represents GalT. The region of interest indicated in (A) was then imaged by confocal (B) and dSTORM imaging (E). ICA images of (B) and (E) are shown in (C) and (F), respectively. Intensity line profiles (D) and (G) taken from (B) and (E), respectively, illustrate the different spatial resolution. Scale bars represent 3 μ m (A) and 600 nm (B-D). (C) and (F) show ICA images of (B) and (E) respectively, where yellow pixels show co-localization and blue pixels show anti-co-localization.

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of *d*STORM (E) compared with confocal imaging (Fig 2A, B). An intensity line profile illustrates this increase in more detail. The spatial distribution was further analyzed to evaluate the co-localization of the *cis*- and *trans*-Golgi markers. The increased spatial resolution now enables a more refined analysis of whether two proteins co-localize. To quantify co-localization, we correlated both imaging channels pixel-wise and calculated the Pearson coefficient (intensity correlation analysis, ICA) (18). Positive values report a co-localization of two proteins within a particular pixel. For the confocal image (Figure 2B), ICA (Figure 2C) 11.4% of pixels showed co-localization (corresponding to yellow pixels in Figure 2B). The same analysis on the super-resolution image of the same region (Figure 2E, F) resulted in only 4.5% of pixels showing co-localization. As a consequence, the dimensions of regions in which both proteins co-localize are overestimated by confocal microscopy, as a result of the poorer spatial resolution. Super-resolution microscopy provides a more realistic picture of the co-localization of both proteins. In general, co-localization analysis of confocal images over-estimates both the size of co-localization sites and the size of co-localizing structures, even when analyzing discrete structures.

With this in hand, we analyzed the co-localization of the two Golgi-markers under different experimental conditions. Relevant cells (Figure 3) were selected out of hundreds from a widefield screen to be representative and characteristic for the respective condition. The Golgi complex is a rather compact and large structure; the labeled proteins occur in high numbers and were marked with dye conjugated antibodies. We note that the density of fluorophores will be high, so that the amount of photobleaching during widefield screening and confocal imaging will be small and should not affect *d*STORM resolution.

Confocal imaging visualized the partially fragmented Golgi complex, which was observed after 20 min incubation with nocodazole, fairly well by imaging either marker (Figure 3C; see also Figure 1A for widefield). ICA of the confocal image revealed not only more co-localization than in the *d*STORM images (Table 2) but also

Table 2. Co-localization analysis by intensity-based correlation analysis (ICA).

	ICA of confocal images (%)	ICA of <i>d</i> STORM images (%)
Control	9.6	5.5
Nocodazole	7.7	4.2
Brefeldin A	5.5	1.6
The numbers in the table quantify the relative fraction of image pixels showing a co-localization value above zero.		

a broader distribution of the co-localizing pixels in different cells (71% in confocal instead of 4.2% in *d*STORM) (Figure 3C). Furthermore, *d*STORM imaging can resolve GM130 and GalT structural motifs that are hidden or unresolved in the confocal mode

(Figure 3C, 3F). This allows the collection of more reliable information on size, number, and shape of the Golgi ministacks after treatment of cells with nocodazole.

Treatment of cells with Brefeldin A (BFA) induces redistribution of the Golgi

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Figure 3. Distribution of GM130 and GalT under different treatments. GM130 and GalT were imaged in confocal (A, B, C) and dSTORM (D, E, F) mode. Red represents GM130 and green represents GalT. Intact Golgi complexes in the untreated cells are shown in (A) and (D), redistribution of the Golgi complexes after addition of BFA is shown in (B) and (E), fragmentation of the Golgi complex after addition of nocodazole is shown in (C) and (F). The confocal images were analyzed for co-localization using ICA (G, H, I). The ICA analyses of the dSTORM images are shown in the bottom row (J, K, L) (scale bars represent 5 μm (fluorescence images) and 1 μm (co-localization images)). Arrows in (F) indicate fiducial markers within the region of interest used for alignment of the red and green channels. More fiducial markers lie outside of selected regions and are not shown.

complex to the endoplasmic reticulum (ER) (Figure 3B, E) (19,20). In contrast to the experiments with nocodazole, where both GalT and GM130 had a similar localization pattern, GalT and GM130 showed a different behavior after BFA treatment. GalT was re-localized to the ER and formed a more reticular pattern, whereas GM130 appeared at the discrete ER exit sites, perceived as punctuate structures (21–24). Even if the separation of these two localization patterns is easily achieved by a confocal microscope (Figure 3B), *d*STORM provides a more refined distribution, size and number of punctuate GM130 specific structures (Figure 3E). Similar to the experiments with nocodazole, a considerably improved co-localization of both markers is obtained (Table 2). The same is true for the untreated cells with an intact Golgi complex (Figure 3A, D). Spatial resolution of cis- and trans-Golgi markers is increased nearly 2-fold according ICA analysis in *d*STORM imaging compared with confocal images of the same cells (Table 2).

For the intact Golgi complex, co-localization analysis based on confocal images might be even more difficult due to the high densities of the markers (Figure 3A, G). Clearly, *d*STORM in combination with ICA analysis is able to overcome these problems, particularly in the compact regions of the Golgi complex (Figure 3D, J). In addition, single-molecule based super-resolution techniques offer the additional advantage of providing molecular coordinates, which can be further processed in, for example, molecular-level co-localization analysis (13,14). This is significantly different to co-localization analysis in confocal microscopy, which is based on the intensity information in individual pixels and thus is spatially constrained. In a coordinate-based approach applied to single-molecule localization data, a co-localization value can be attributed to each single molecule.

The correlative microscopy approach presented here combines fast widefield imaging of large sample numbers with high- and super-resolution microscopy, providing near-molecular level information on cellular structures. If certain structures of interest found in a widefield screen need further investigation by techniques with high resolution, the best representative

regions can be chosen from the widefield images. The widefield imaging mode is then used as a filter or trigger system in order to identify predefined structures of interest and find the positions that are to be imaged in the other microscopy modes. This would be beneficial in high-throughput screening experiments, where a manual selection of cells for higher resolution imaging becomes quickly impractical due to the huge amount of data. Even in experiments where a sparse phenotype should be relocated and imaged in high-resolution systems, this approach can help by providing an image of the whole sample at low resolution. The high-resolution image can be recorded at exactly the right position by marking the appropriate spot in the low-resolution image. Due to the possibility of performing multiple experiments on the same cell, the information content is expanded, assuming that photobleaching is negligible (e.g., large structures with high concentrations of labeled proteins, short imaging times per region beforehand). Our workflow for relocating cells on different microscopic systems is furthermore not restricted to the fluorescence microscopic methods presented here, but is also applicable for the integration of any microscopic technique where the position of the reference markers can be identified.

Author contributions

The conception of the study, writing, and editing of the manuscript were done by all authors; the development of integrative microscopy approach and assay adaptation were done by B.F., M.G., J.R., T.L., V.S., M.H., and H.E. The study was executed by B.F., M.G., T.L., and J.R., ICA analysis of *d*STORM images was performed by B.F.

Acknowledgments

We thank Sebastian Malkusch for help with co-localization analysis. This work was supported by contract research “Methoden für die Lebenswissenschaften” of the Baden-Württemberg Stiftung (grant nr. P-LS-SPII/11) and by the “New methods in systems biology” program of the Federal Ministry of Education and Research (grant nr. 0315523A). The

ViroQuant-CellNetworks RNAi screening facility is supported by the CellNetworks-Cluster of Excellence (EXC81).

Competing interests

The authors declare no competing interests.

References

1. Collinet, C., M. Stoter, C.R. Bradshaw, N. Samusik, J.C. Rink, D. Kenski, B. Habermann, F. Buchholz, et al. 2010. Systems survey of endocytosis by multiparametric image analysis. *Nature* 464:243–249.
2. Neumann, B., T. Walter, J.-K. Hériché, J. Bulkescher, H. Erfle, C. Conrad, P. Rogers, I. Poser, et al. 2010. Phenotypic profiling of the human genome by time-lapse microscopy reveals cell division genes. *Nature* 464:721–727.
3. Osterwald, S., S. Wörz, J. Reymann, F. Sieckmann, K. Rohr, H. Erfle, and K. Rippe. 2011. A three-dimensional co-localization RNA interference screening platform to elucidate the alternative lengthening of telomeres pathway. *Biotechnology Journal*.
4. Conrad, C., A. Wünsche, T.H. Tan, J. Bulkescher, F. Sieckmann, F. Verissimo, A. Edelstein, T. Walter, et al. 2011. Micro-pilot: automation of fluorescence microscopy-based imaging for systems biology. *Nat. Methods* 8:246–249.
5. Hell, S.W. 2007. Far-field optical nanoscopy. *Science* 316:1153–1158.
6. Heilemann, M. 2010. Fluorescence microscopy beyond the diffraction limit. *J. Biotechnol.* 149:243–251.
7. Galbraith, C.G. and J.A. Galbraith. 2011. Super-resolution microscopy at a glance. *J. Cell Sci.* 124:1607–1611.
8. Betzig, E., G.H. Patterson, R. Sougrat, O.W. Lindwasser, S. Olenych, J.S. Bonifacino, M.W. Davidson, J. Lippincott-Schwartz, and H.F. Hess. 2006. Imaging intracellular fluorescent proteins at nanometer resolution. *Science* 313:1642–1645.
9. Rust, M.J., M. Bates, and X. Zhuang. 2006. Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM). *Nat. Methods* 3:793–795.
10. Fölling, J., M.L. Bossi, H. Bock, R. Medda, C.A. Wurm, B. Hein, S. Jakobs,

- C. Eggeling, and S.W. Hell. 2008. Fluorescence nanoscopy by ground-state depletion and single-molecule return. *Nat. Methods* 5:943-945.
11. Heilemann, M., S. van de Linde, M. Schüttelpelz, R. Kasper, B. Seefeldt, A. Mukherjee, P. Tinnefeld, and M. Sauer. 2008. Subdiffraction-resolution fluorescence imaging with conventional fluorescent probes. *Angew. Chem. Int. Ed. Engl.* 47:6172-6176.
 12. Lemmer, P., M. Gunkel, D. Baddeley, R. Kaufmann, A. Urich, Y. Weiland, J. Reyman, P. Müller, et al. 2008. SPDM: light microscopy with single-molecule resolution at the nanoscale. *Appl. Phys. B* 93:1-12.
 13. Malkusch, S., U. Endesfelder, J. Mondry, M. Gelléri, P.J. Verveer, and M. Heilemann. 2012. Coordinate-based co-localization analysis of single-molecule localization microscopy data. *Histochem. Cell Biol.* 137:1-10.
 14. Gunkel, M., F. Erdel, K. Rippe, P. Lemmer, R. Kaufmann, C. Hörmann, R. Amberger, and C. Cremer. 2009. Dual color localization microscopy of cellular nanostructures. *Biotechnol. J.* 4:927-938.
 15. Wolter, S., M. Schüttelpelz, M. Tscherepanow, S. van de Linde, M. Heilemann, and M. Sauer. 2010. Real-time computation of subdiffraction-resolution fluorescence images. *J. Microsc.* 237:12-22.
 16. Nakamura, N., C. Rabouille, R. Watson, T. Nilsson, N. Hui, P. Slusarewicz, T.E. Kreis, and G. Warren. 1995. Characterization of a cis-Golgi matrix protein, GM130. *J. Cell Biol.* 131:1715-1726.
 17. Röttger, S., J. White, H.H. Wandall, J.C. Olivo, A. Stark, E.P. Bennett, C. Whitehouse, E.G. Berger, et al. 1998. Localization of three human polypeptide GalNAc-transferases in HeLa cells suggests initiation of O-linked glycosylation throughout the Golgi apparatus. *J. Cell Sci.* 111:45-60.
 18. Li, Q., A. Lau, T.J. Morris, L. Guo, C.B. Fordyce, and E.F. Stanley. 2004. A Syntaxin 1, Gαo, and N-Type Calcium Channel Complex at a Presynaptic Nerve Terminal: Analysis by Quantitative Immunocolocalization. *J. Neurosci.* 24:4070-4081.
 19. Klausner, R. and J. Donaldson. 1992. Brefeldin A: insights into the control of membrane traffic and organelle structure. *The Journal of cell ...*
 20. Lippincott-Schwartz, J., L.C. Yuan, J.S. Bonifacino, and R.D. Klausner. 1989. Rapid redistribution of Golgi proteins into the ER in cells treated with brefeldin A: evidence for membrane cycling from Golgi to ER. *Cell* 56:801-813.
 21. Tang, B., S. Low, and W. Hong. 1995. Differential response of resident proteins and cycling proteins of the Golgi to brefeldin A. - Abstract - UK PubMed Central. *European journal of cell biology.*
 22. Mardones, G.A., C.M. Snyder, and K.E. Howell. 2006. Cis-Golgi matrix proteins move directly to endoplasmic reticulum exit sites by association with tubules. *Mol. Biol. Cell* 17:525-538.
 23. Miles, S. 2001. Evidence that the entire Golgi apparatus cycles in interphase HeLa cells: sensitivity of Golgi matrix proteins to an ER exit block. *J. Cell Biol.* 155:543-556.
 24. Altan-Bonnet, N., R. Sougrat, W. Liu, E.L. Snapp, T. Ward, and J. Lippincott-Schwartz. 2006. Golgi inheritance in mammalian cells is mediated through endoplasmic reticulum export activities. *Mol. Biol. Cell* 17:990-1005.

Received 28 June 2013; accepted 10 October 2013.

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